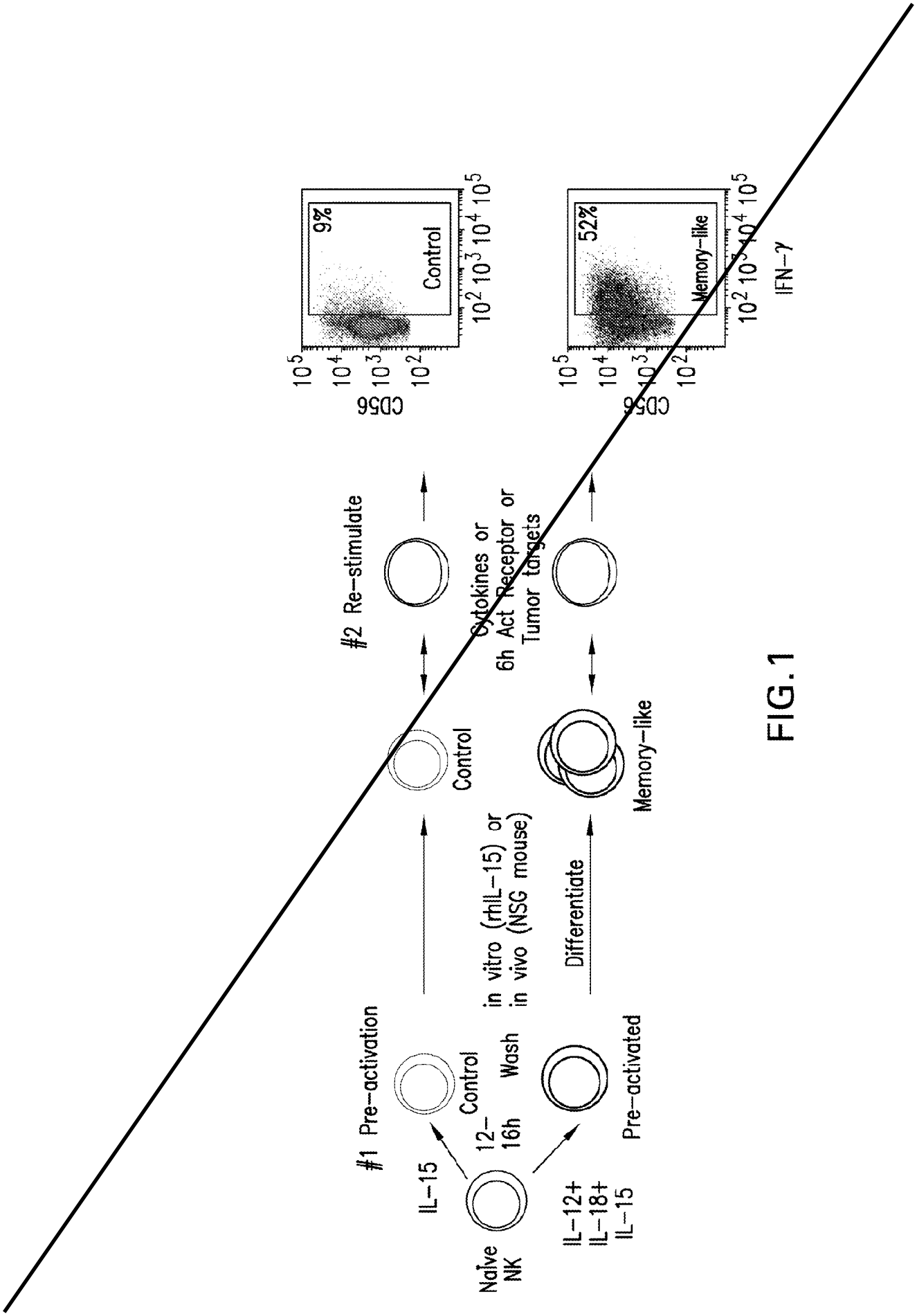

Naïve NK
IL-12+ IL-18+ IL-15
Pre-activated
IL-15
#1 Pre-activation
12-16h
Control
Wash
in vitro (rhIL-15) or in vivo (NSG mouse)
Differentiate
Memory-like
Control
#2 Re-stimulate
Cytokines or 6h Act Receptor or Tumor targets
Memory-like
Control
52%
9%
CD56
IFN-γ

FIG. 1

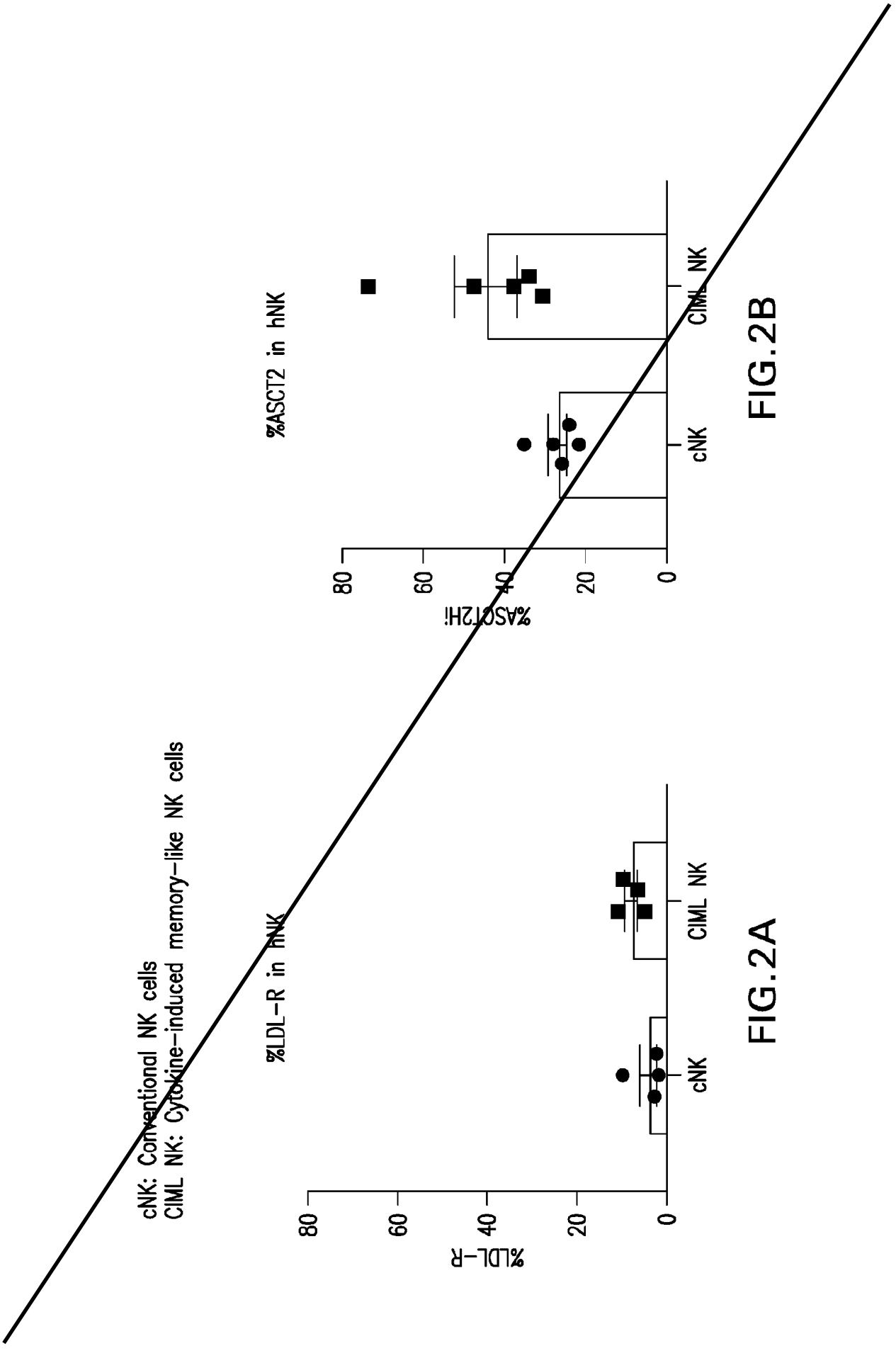

cNK: Conventional NK cells
CIML NK: Cytokine-induced memory-like NK cells
%LDL-R in hNK
%LDL-R
0
20
40
60
80
cNK
CIML NK
%ASCT2 in hNK
%ASCT2Hi
0
20
40
60
80
cNK
CIML NK

FIG.2A

FIG.2B

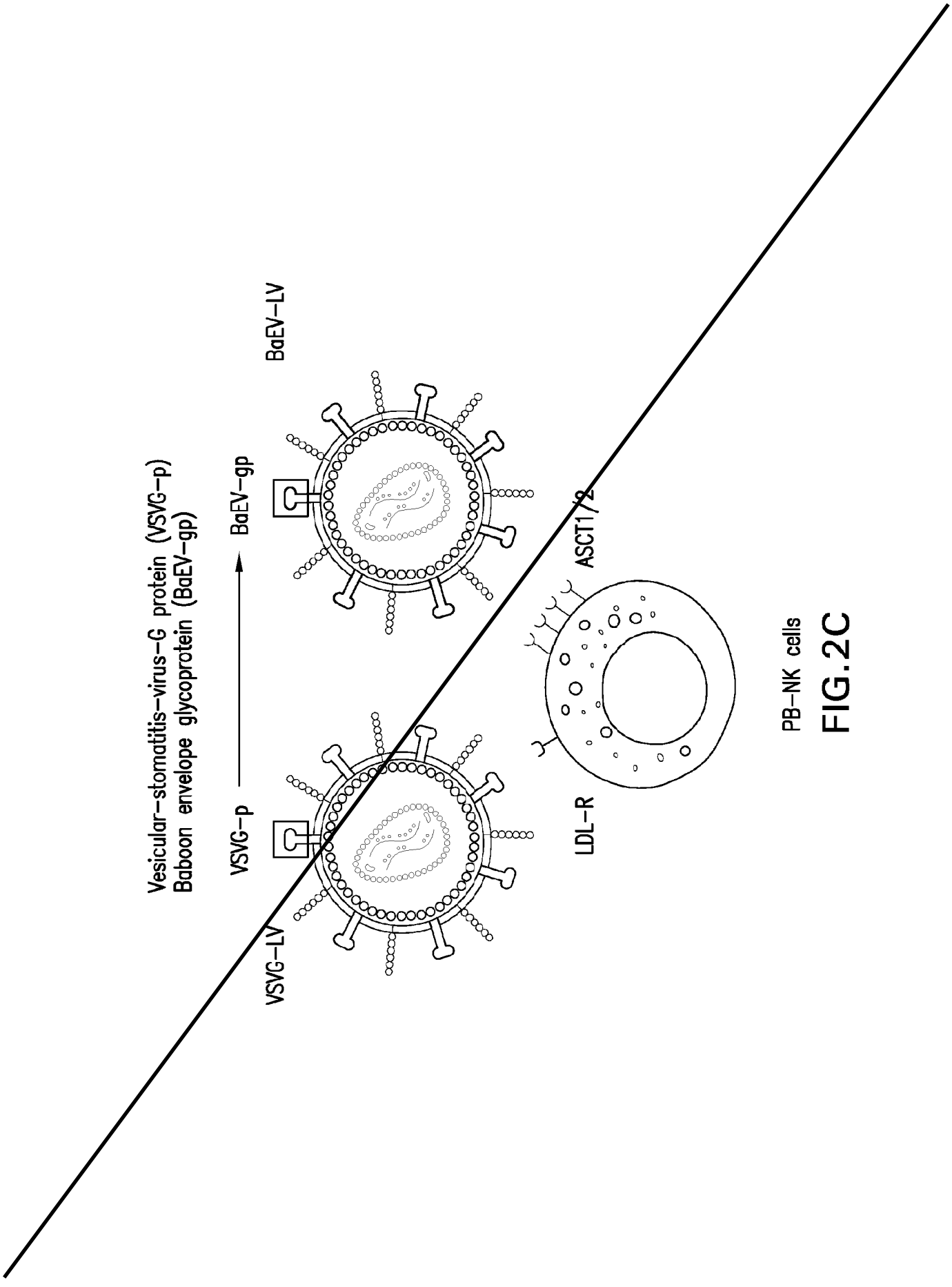

Vesicular-stomatitis-virus-G protein (VSVG-p)
Baboon envelope glycoprotein (BaEV-gp)
VSVG-p
BaEV-gp
BaEV-LV
VSVG-LV
ASCT1/2
LDL-R
PB-NK cells

FIG.2C

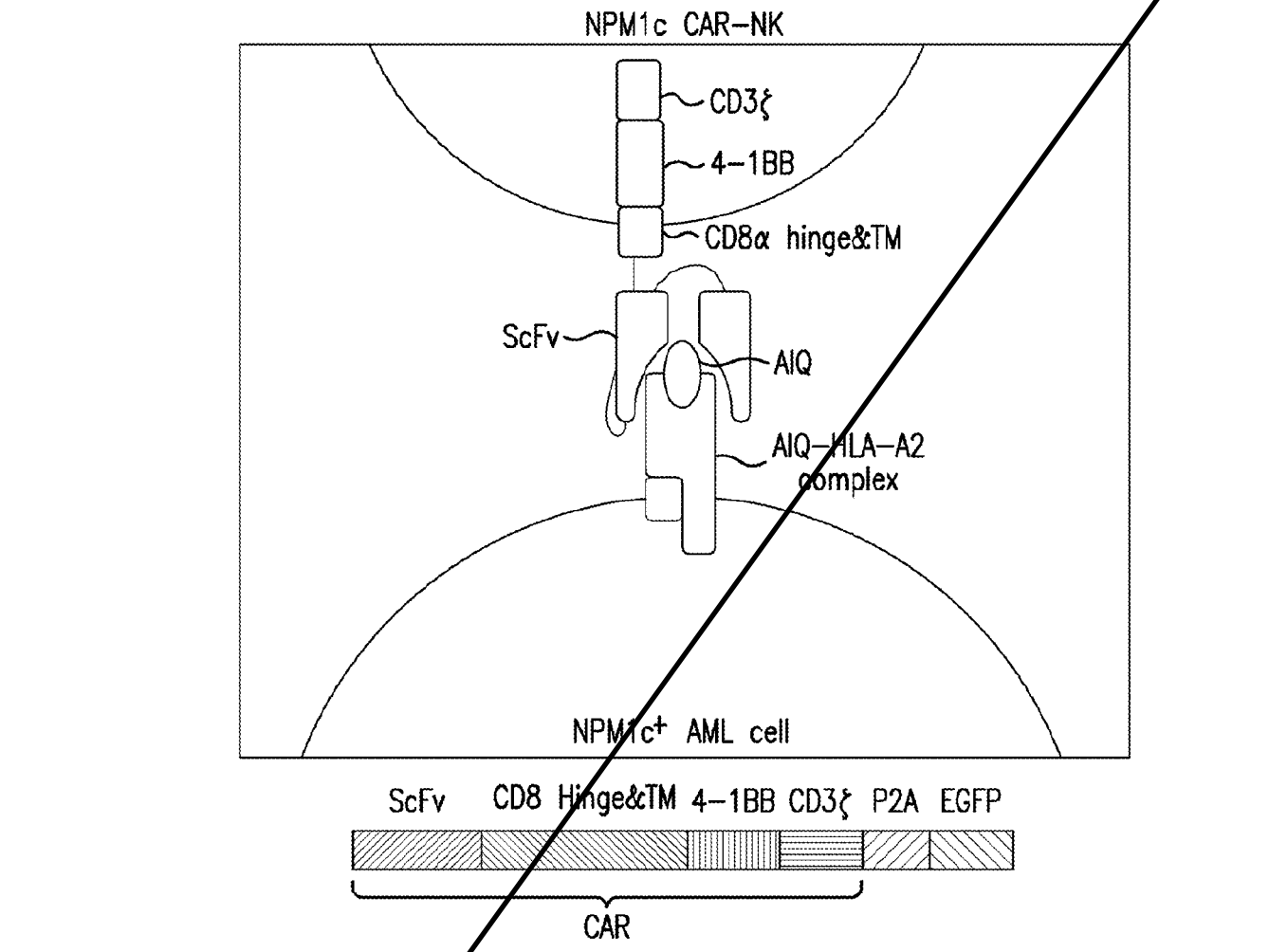

NPM1c CAR-NK
CD3ζ
4-1BB
CD8α hinge&TM
ScFv
AIQ
AIQ-HLA-A2 complex
NPM1c+ AML cell
ScFv CD8 Hinge&TM 4-1BB CD3ζ P2A EGFP
CAR

FIG.3A

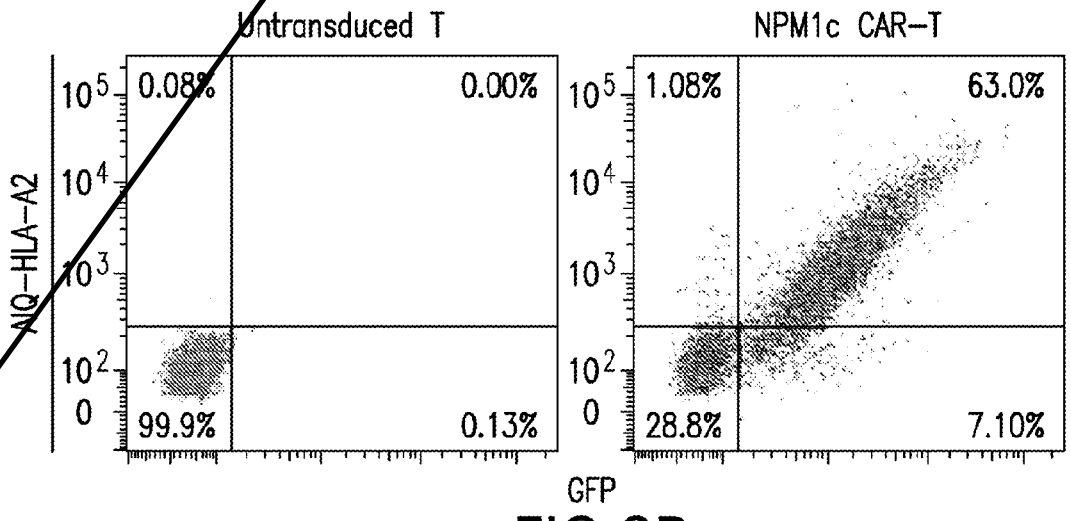

Untransduced T
NPM1c CAR-T
AIQ-HLA-A2
GFP
0.08%
0.00%
99.9%
0.13%
1.08%
63.0%
28.8%
7.10%

FIG.3B

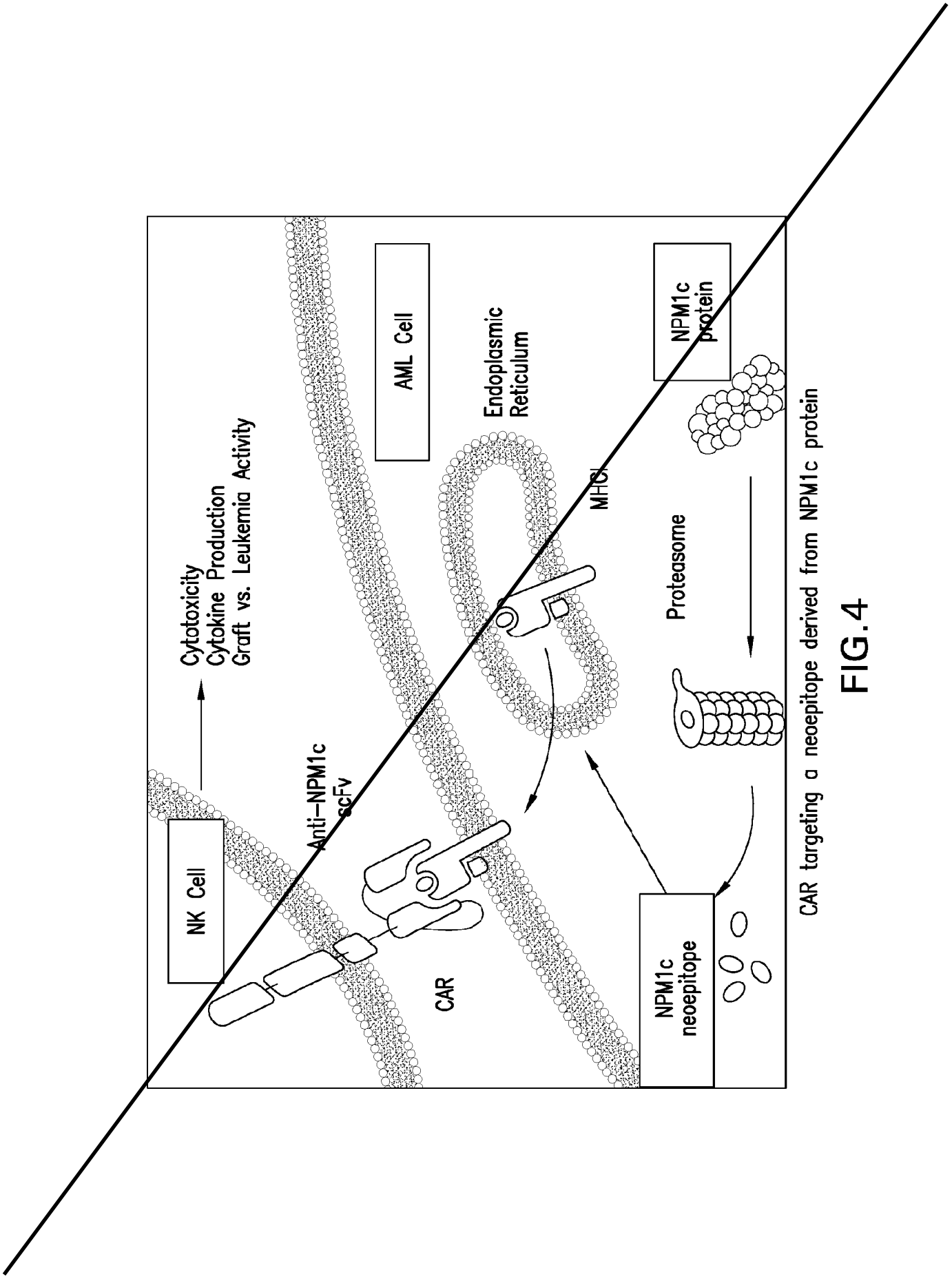

NK Cell
Anti-NPM1c scFv
CAR
AML Cell
Endoplasmic Reticulum
MHCI
NPM1c protein
Proteasome
NPM1c neoepitope
Cytotoxicity
Cytokine Production
Graft vs. Leukemia Activity
CAR targeting a neoepitope derived from NPM1c protein

FIG.4

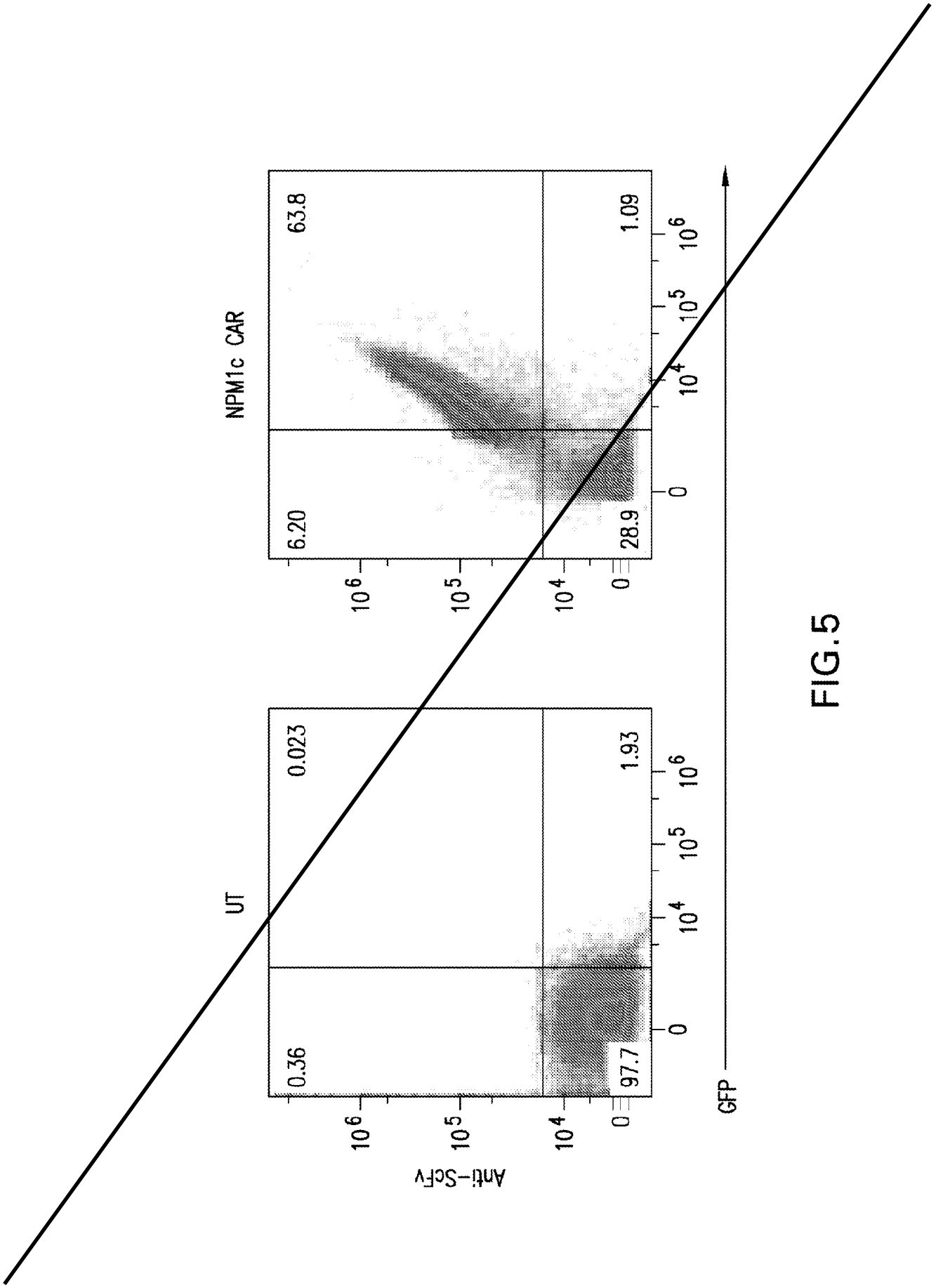

NPM1c CAR
UT
Anti-ScFv
GFP
63.8
1.09
6.20
28.9
0.023
1.93
0.36
97.7
0
10^4
10^5
10^6

FIG.5

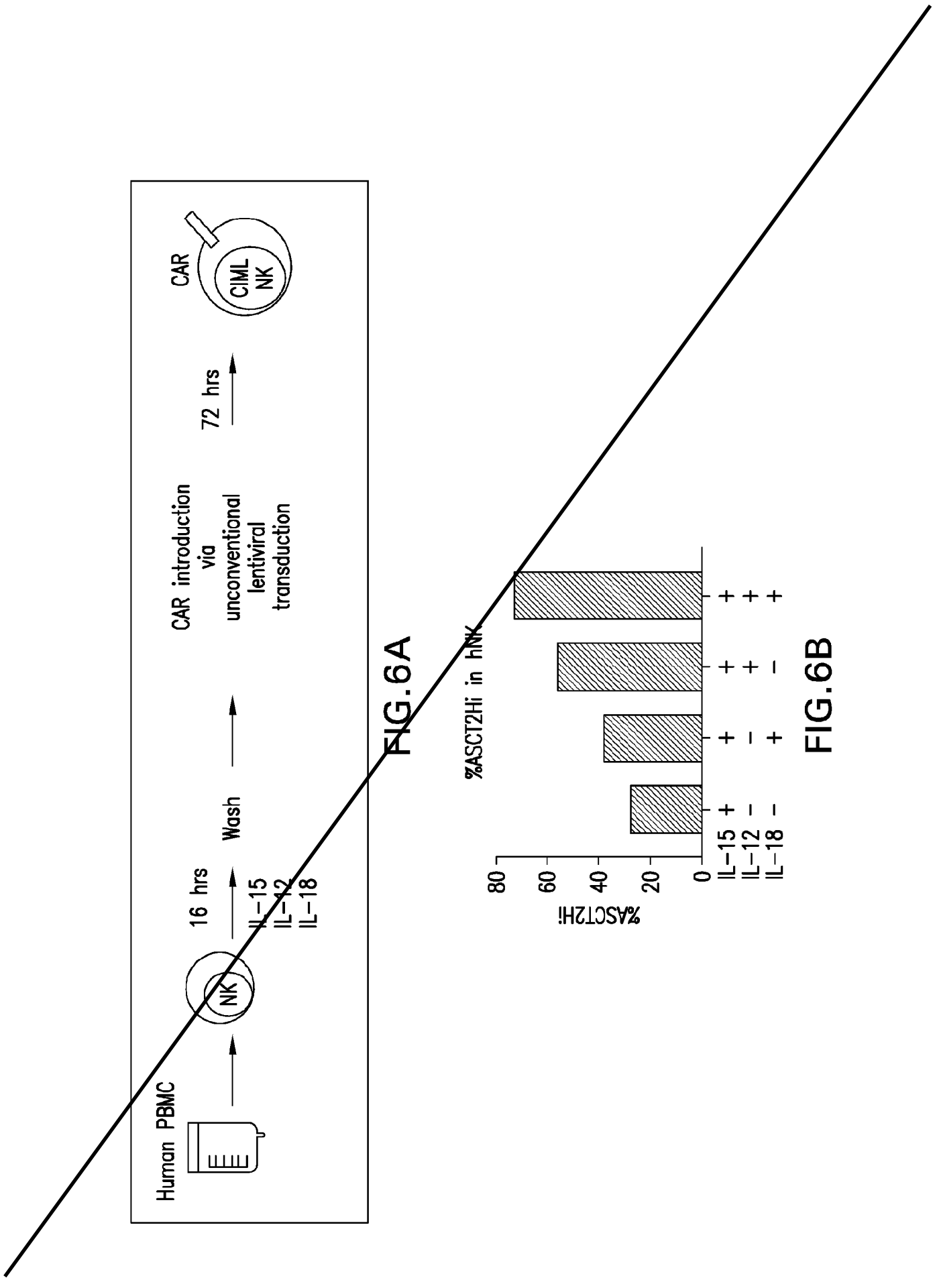

Human PBMC
NK
16 hrs
IL-15
IL-12
IL-18
Wash
CAR introduction via unconventional lentiviral transduction
72 hrs
CAR
CIML NK

FIG.6A

%ASCT2Hi in hNK
%ASCT2Hi
80
60
40
20
0
IL-15 + + + +
IL-12 - + + -
IL-18 - + + +

FIG.6B

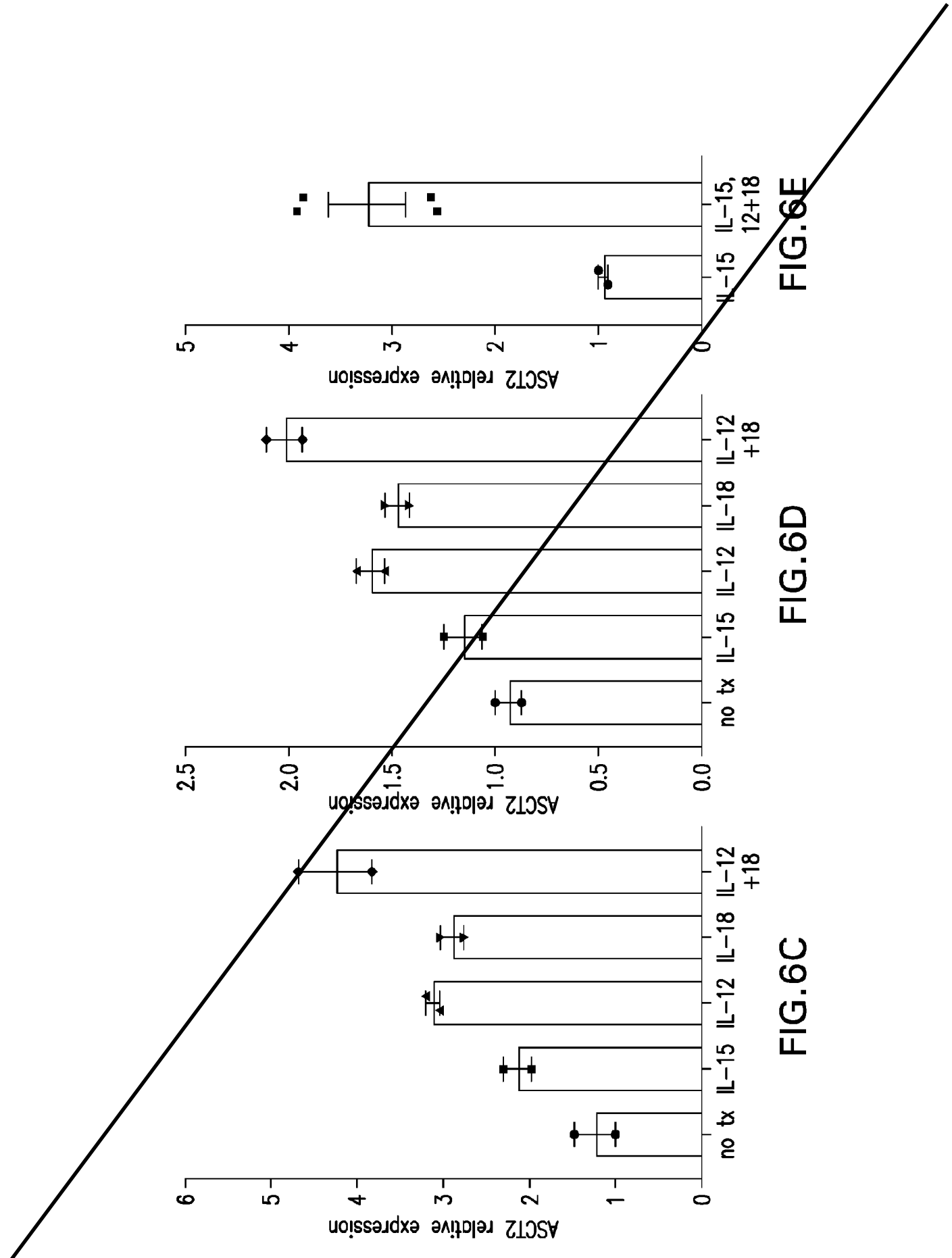


FIG.6C

FIG.6D

FIG.6E

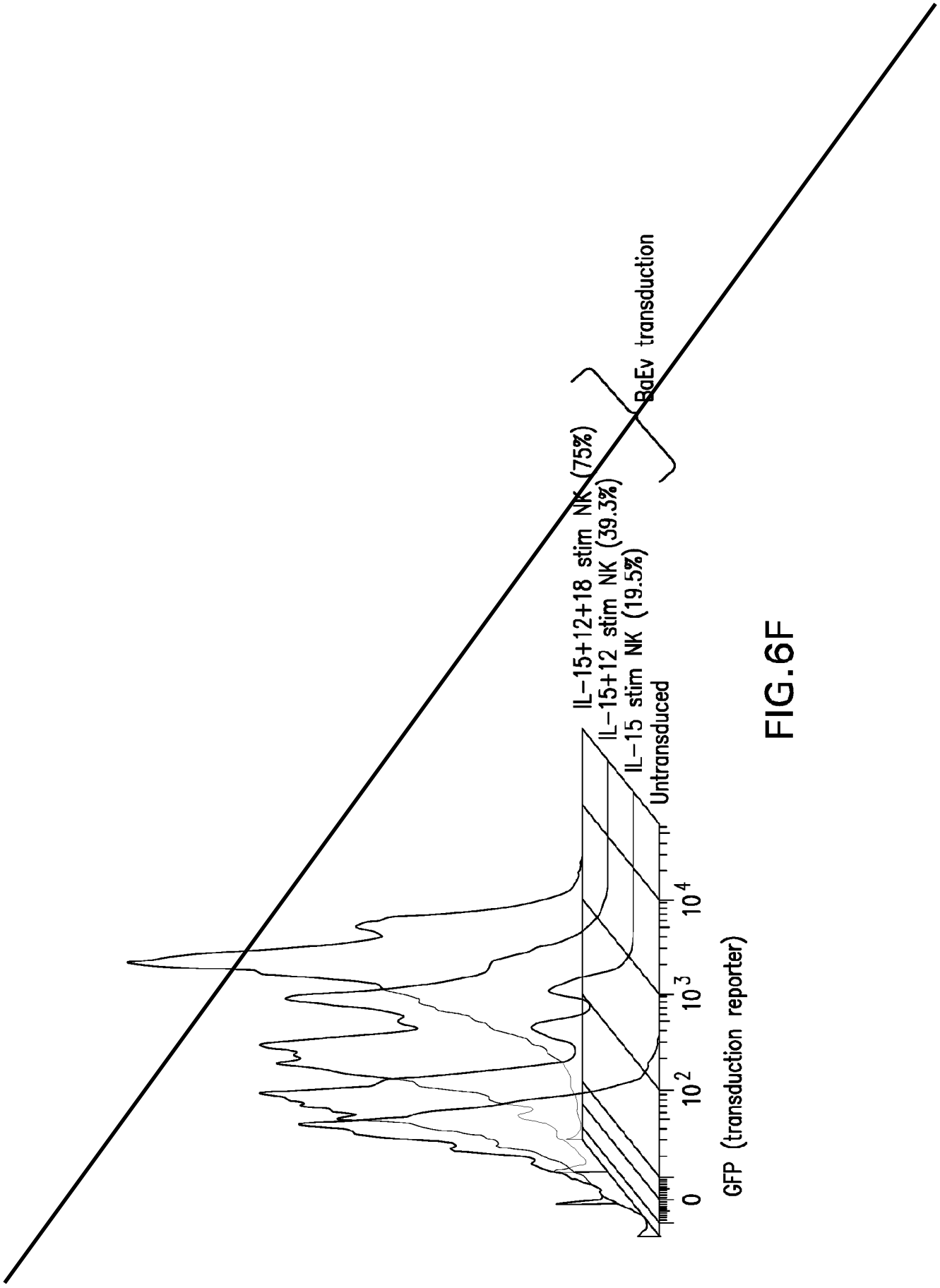

RaEv transduction
IL-15+12+18 stim NK (75%)
IL-15+12 stim NK (39.3%)
IL-15 stim NK (19.5%)
Untransduced
0
10^2
10^3
10^4
GFP (transduction reporter)

FIG.6F

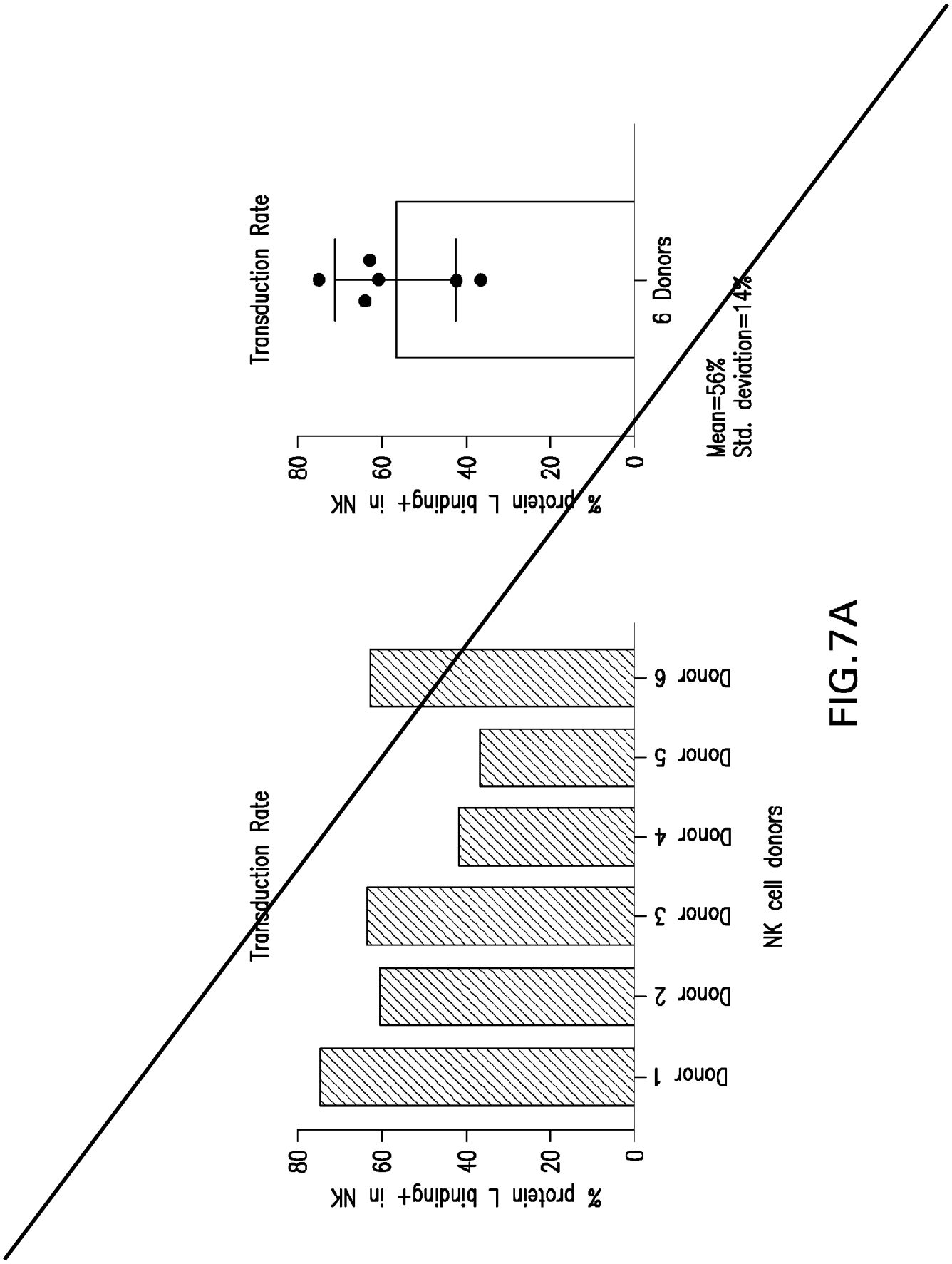

Transduction Rate
% protein L binding+ in NK
0
20
40
60
80
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
NK cell donors
Transduction Rate
% protein L binding+ in NK
0
20
40
60
80
6 Donors
Mean=56%
Std. deviation=14%

FIG.7A

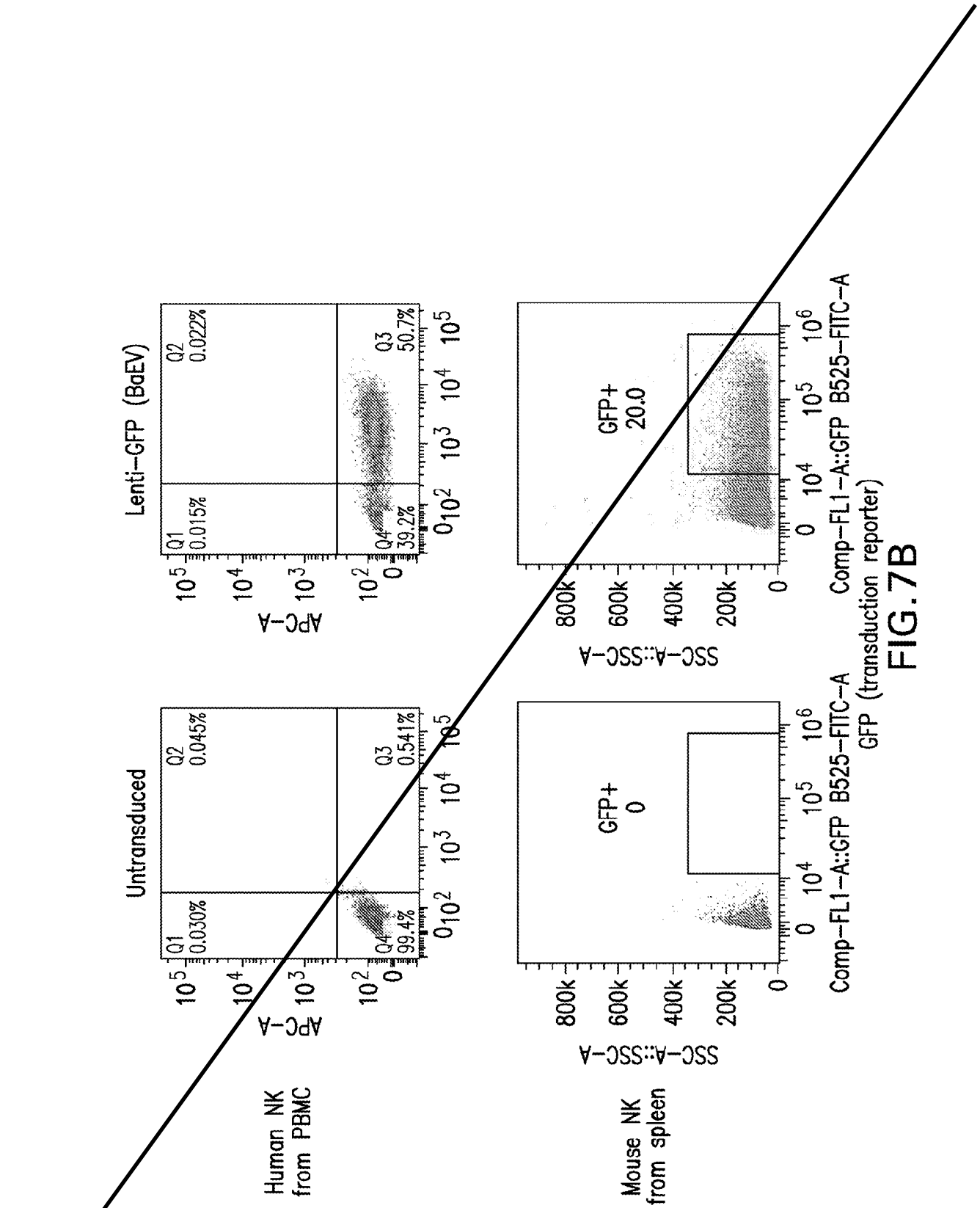

Human NK from PBMC
Mouse NK from spleen
Untransduced
Lenti-GFP (BaEV)
APC-A
SSC-A::SSC-A
Comp-FL1-A::GFP B525-FITC-A
GFP (transduction reporter)
Q1 0.030%
Q2 0.045%
Q4 99.4%
Q3 0.541%
Q1 0.015%
Q2 0.022%
Q4 39.2%
Q3 50.7%
GFP+ 0
GFP+ 20.0

FIG. 7B

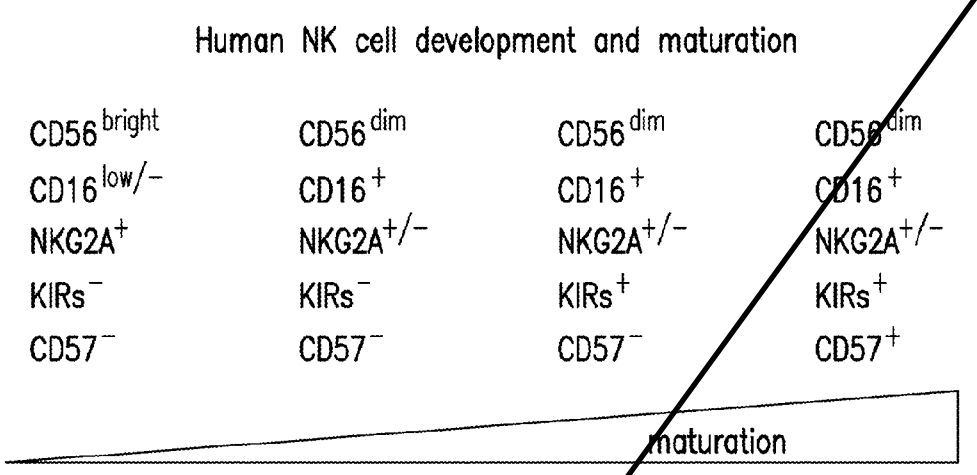


FIG.7C

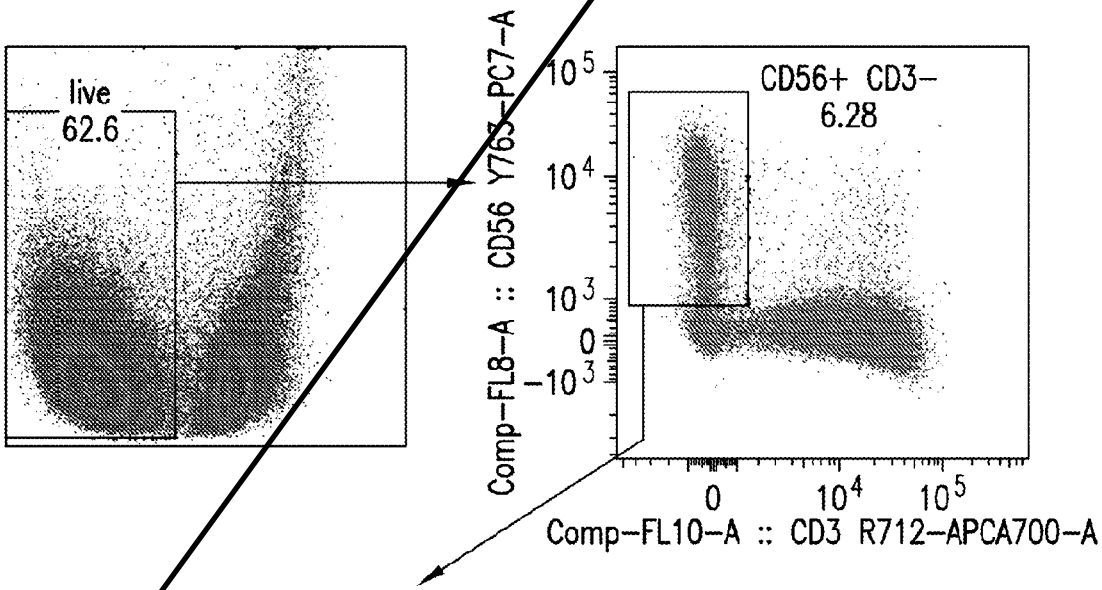


FIG.7D cont. on sheet 13/51

FIG.7D

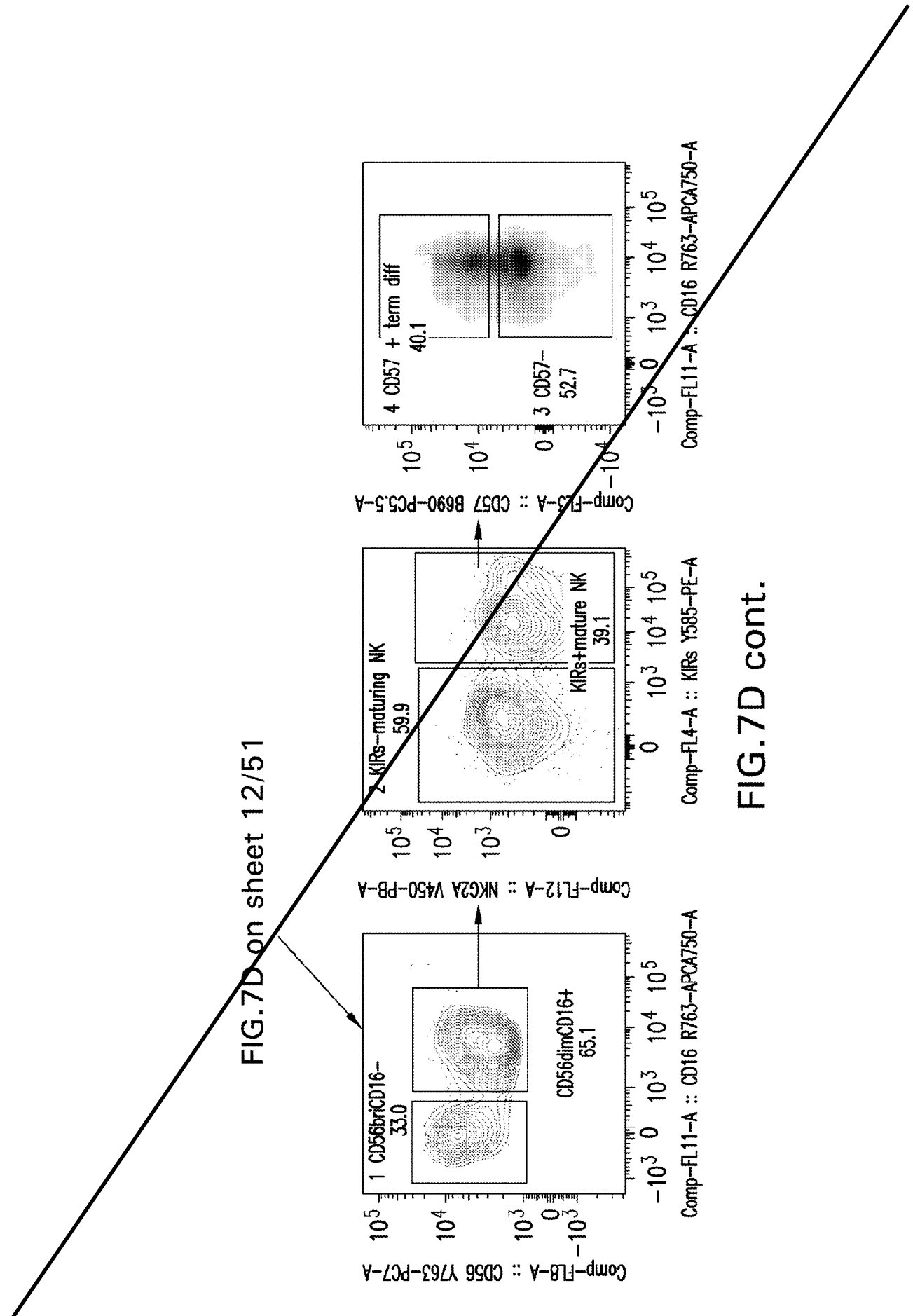

FIG.7D on sheet 12/51
1 CD56briCD16- 33.0
CD56dimCD16+ 65.1
Comp-FL8-A :: CD56 Y763-PC7-A
Comp-FL11-A :: CD16 R763-APCA750-A
Comp-FL12-A :: NKG2A V450-PB-A
2 KIRs-maturing NK 59.9
KIRs+mature NK 39.1
Comp-FL4-A :: KIRs Y585-PE-A
Comp-FL5-A :: CD57 B690-PC5.5-A
4 CD57 + term diff 40.1
3 CD57- 52.7
Comp-FL11-A :: CD16 R763-APCA750-A

FIG.7D cont.

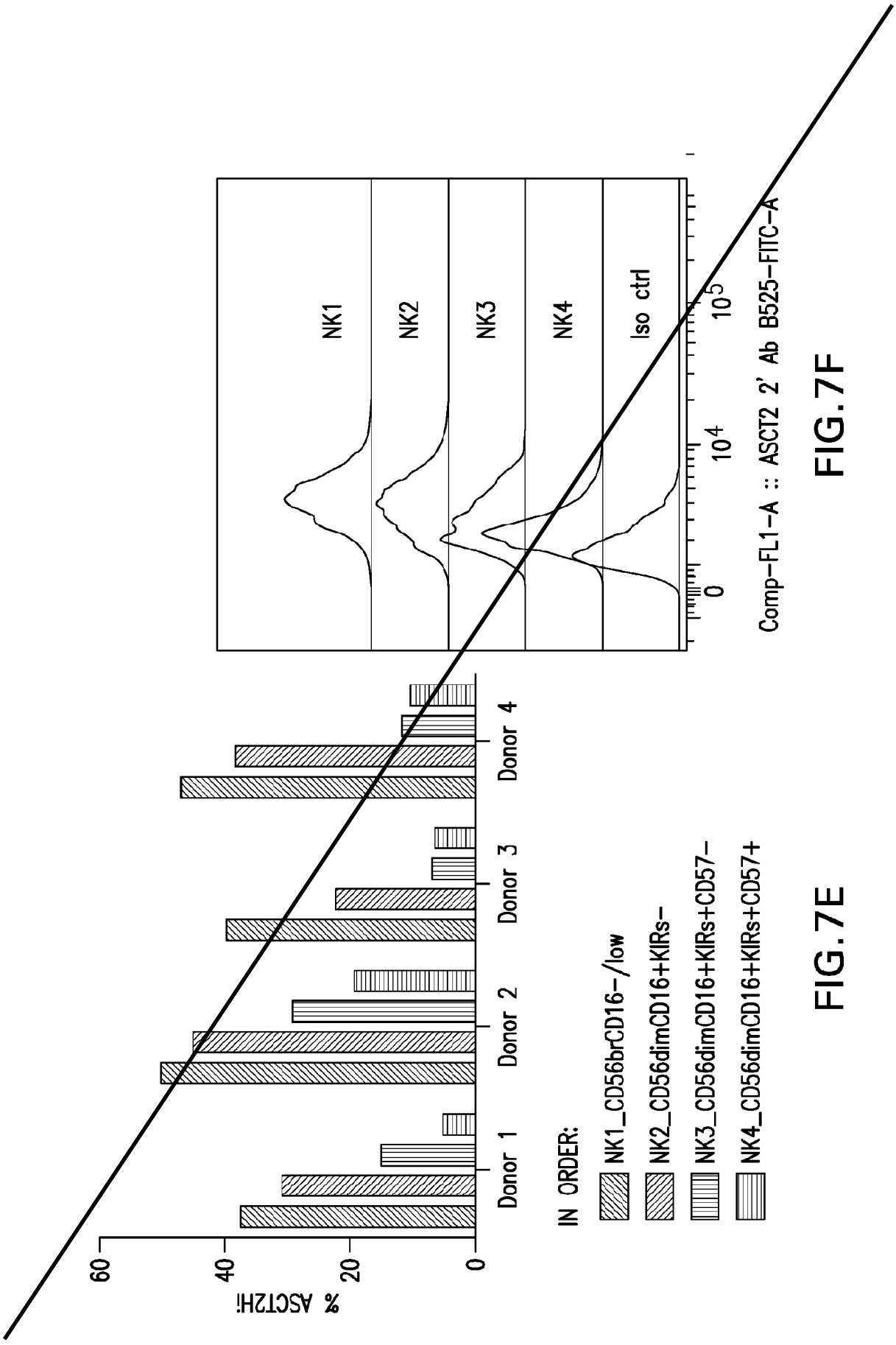

% ASCT2Hi
0
20
40
60
Donor 1
Donor 2
Donor 3
Donor 4
IN ORDER:
NK1_CD56brCD16-/low
NK2_CD56dimCD16+KIRs-
NK3_CD56dimCD16+KIRs+CD57-
NK4_CD56dimCD16+KIRs+CD57+
NK1
NK2
NK3
NK4
Iso ctrl
0
10^4
10^5
Comp-FL1-A :: ASCT2 2' Ab B525-FITC-A

FIG.7E

FIG.7F

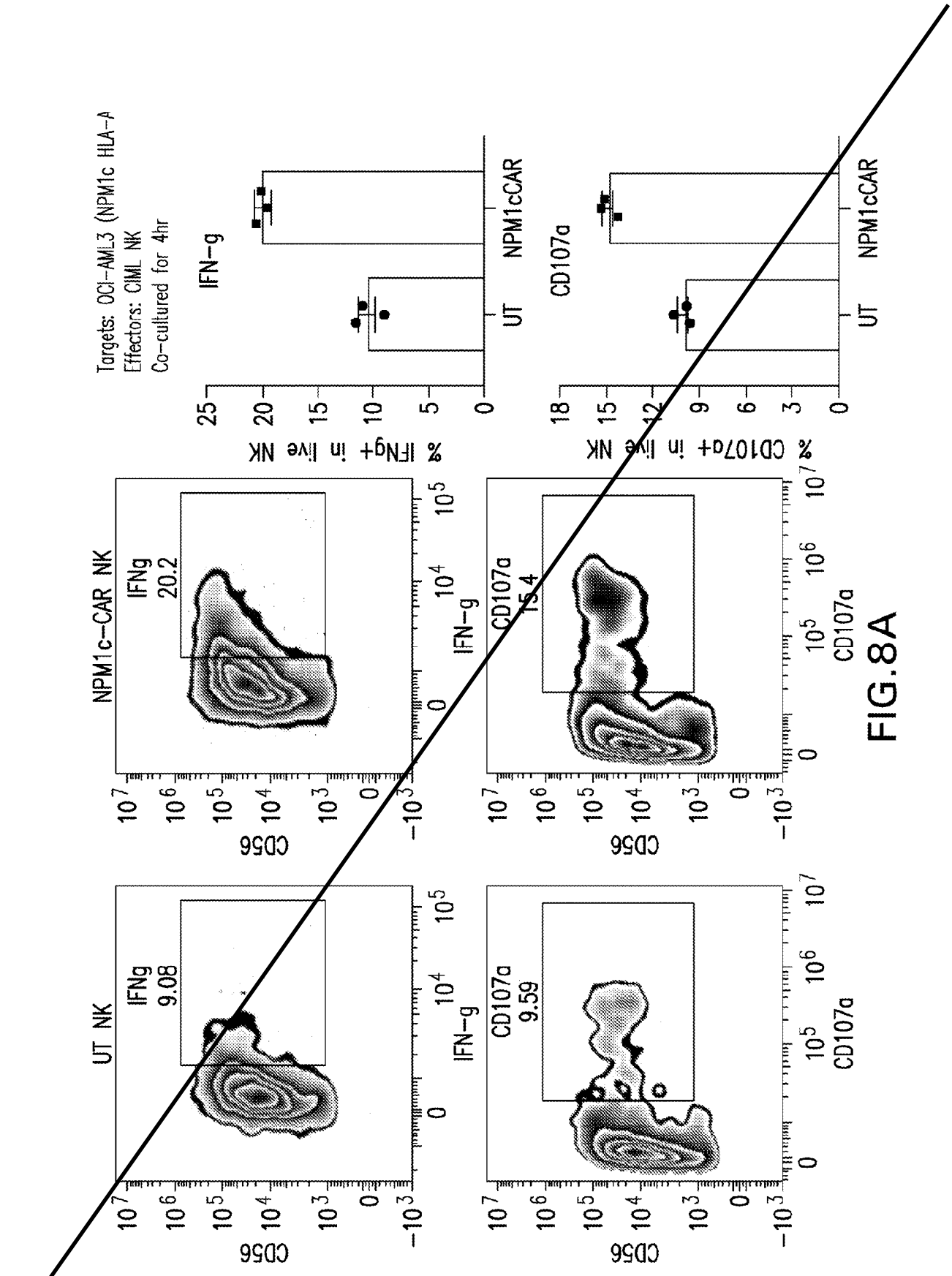

Targets: OCI-AML3 (NPM1c HLA-A
Effectors: CIML NK
Co-cultured for 4hr
IFN-g
% IFNg+ in live NK
UT
NPM1cCAR
CD107a
% CD107a+ in live NK
UT NK
IFNg
9.08
NPM1c-CAR NK
IFNg
20.2
CD107a
9.59
CD107a
15.4
CD56
IFN-g
CD107a

FIG.8A

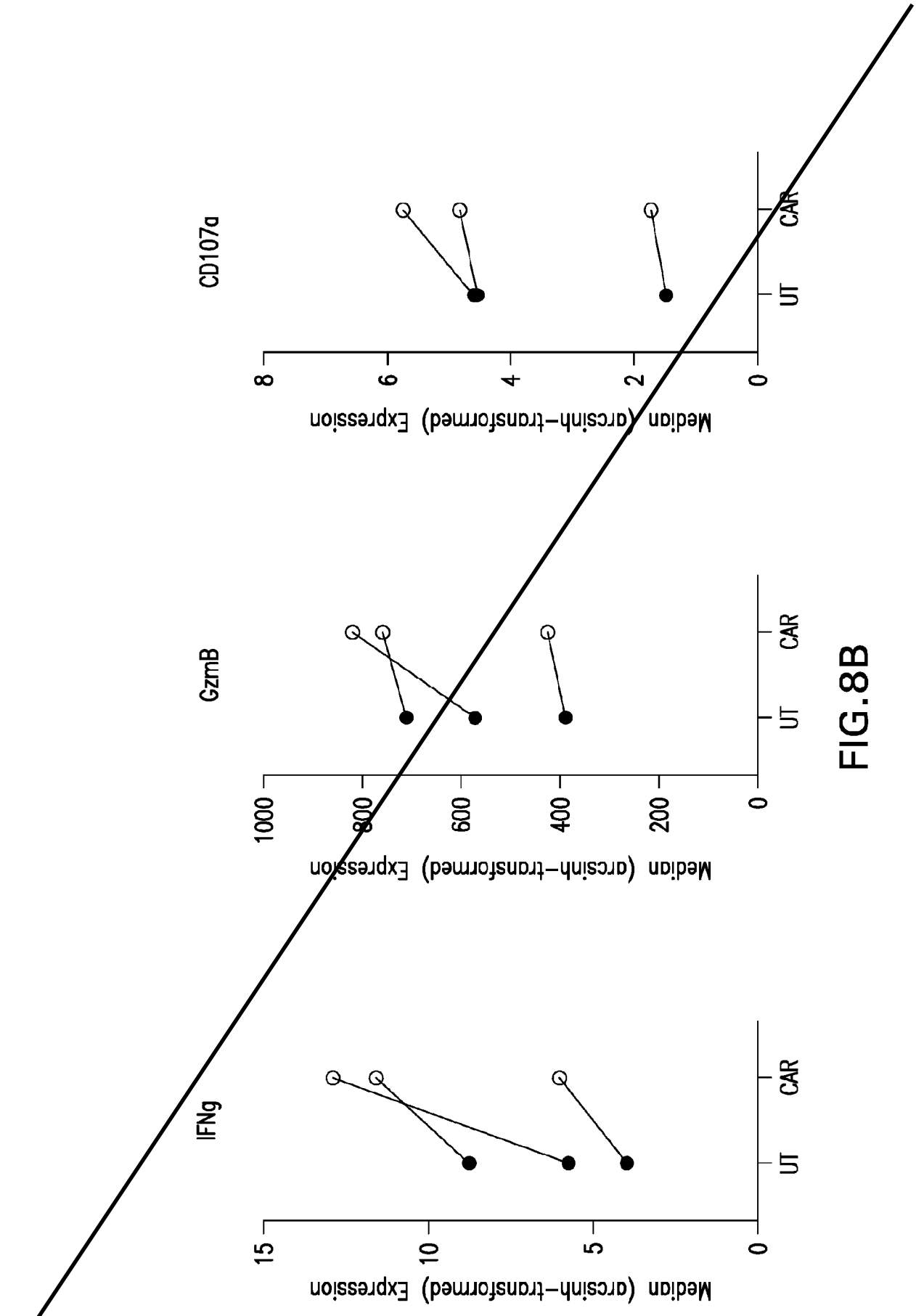

IFNg
GzmB
CD107a
Median (arcsinh−transformed) Expression
0
5
10
15
200
400
600
800
1000
2
4
6
8
UT
CAR

FIG.8B

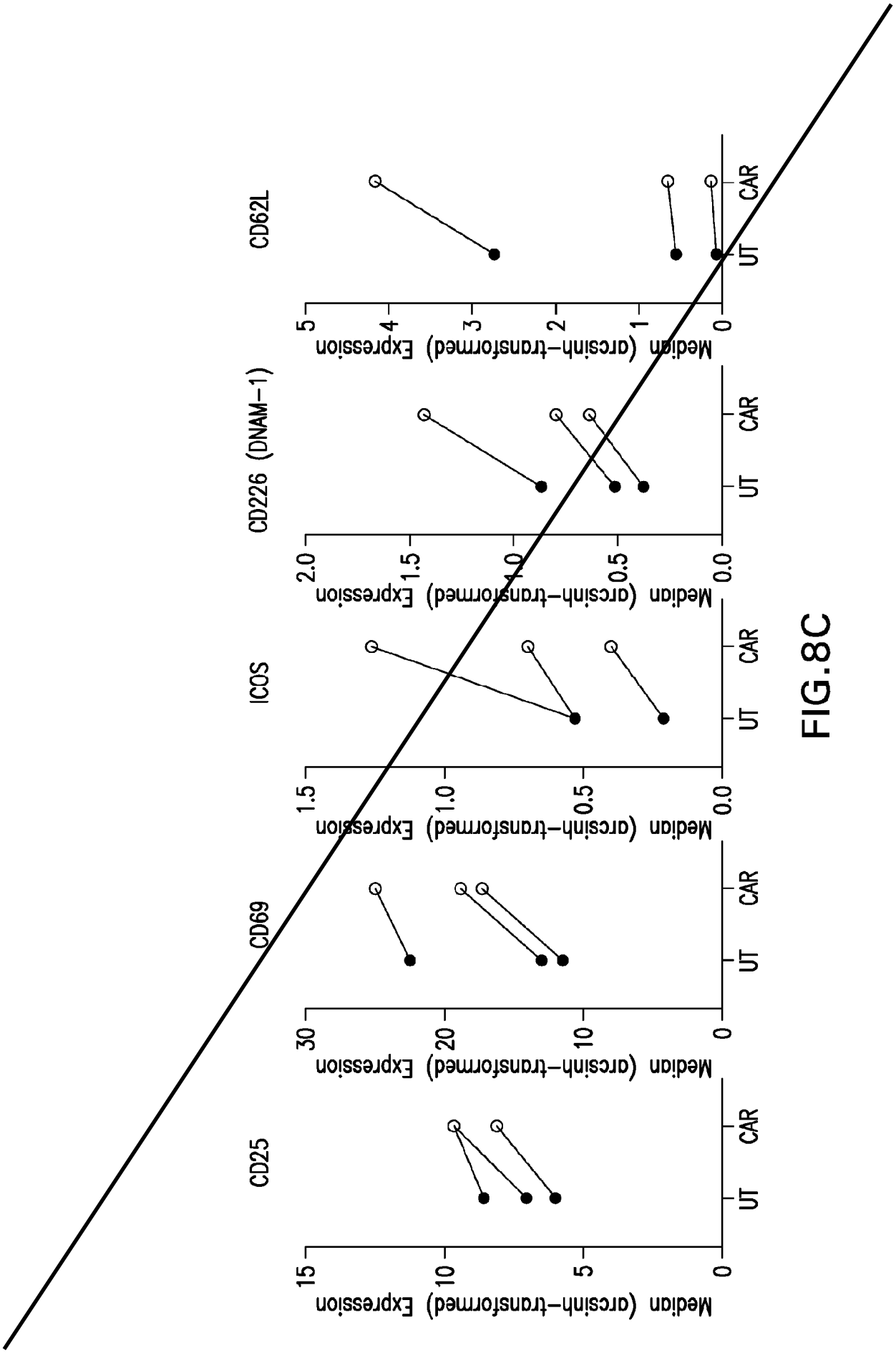

CD25
CD69
ICOS
CD226 (DNAM−1)
CD62L
Median (arcsinh−transformed) Expression
UT
CAR

FIG.8C

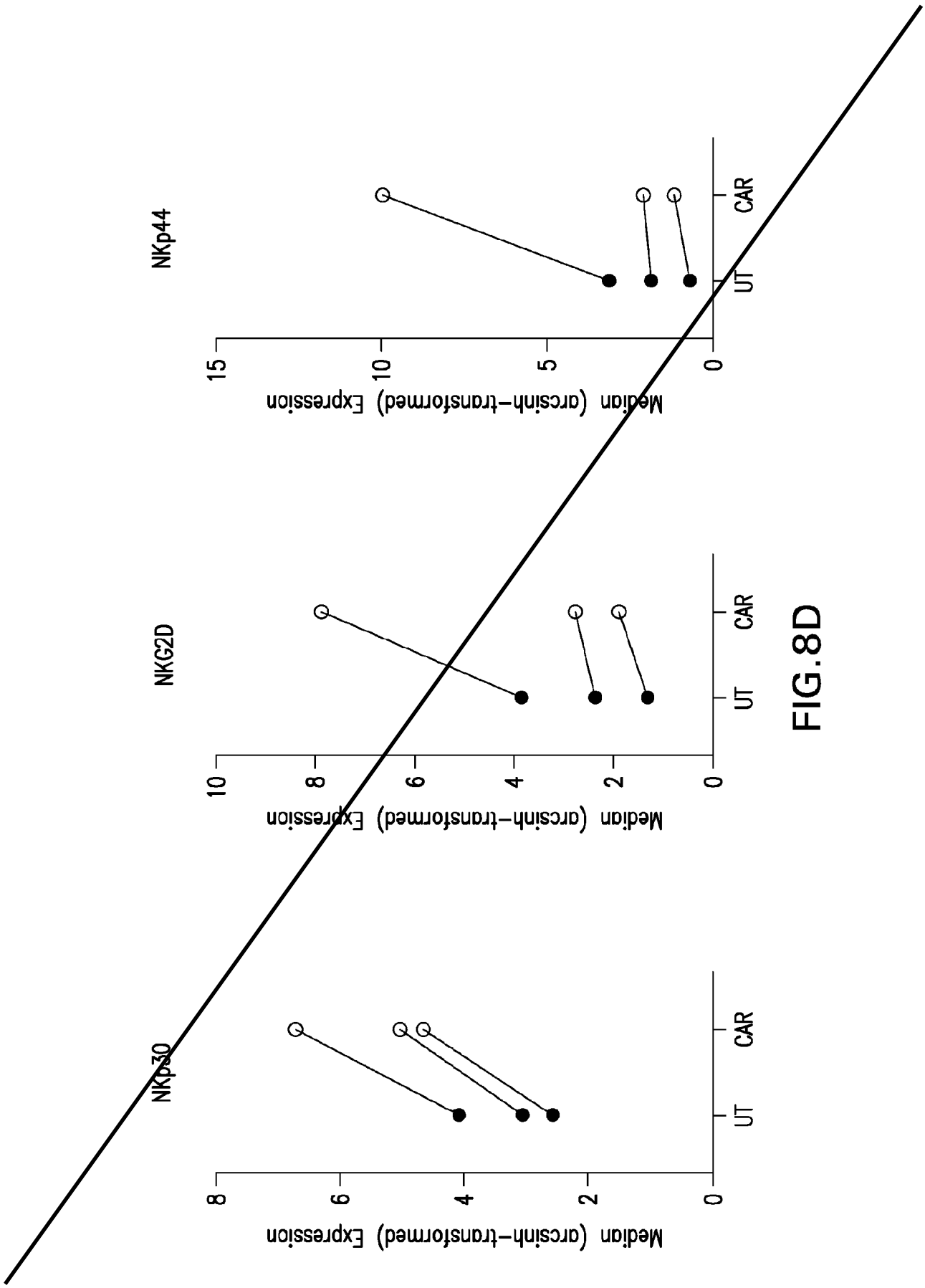

NKp30
NKG2D
NKp44
Median (arcsinh−transformed) Expression
0
2
4
6
8
10
15
UT
CAR

FIG.8D

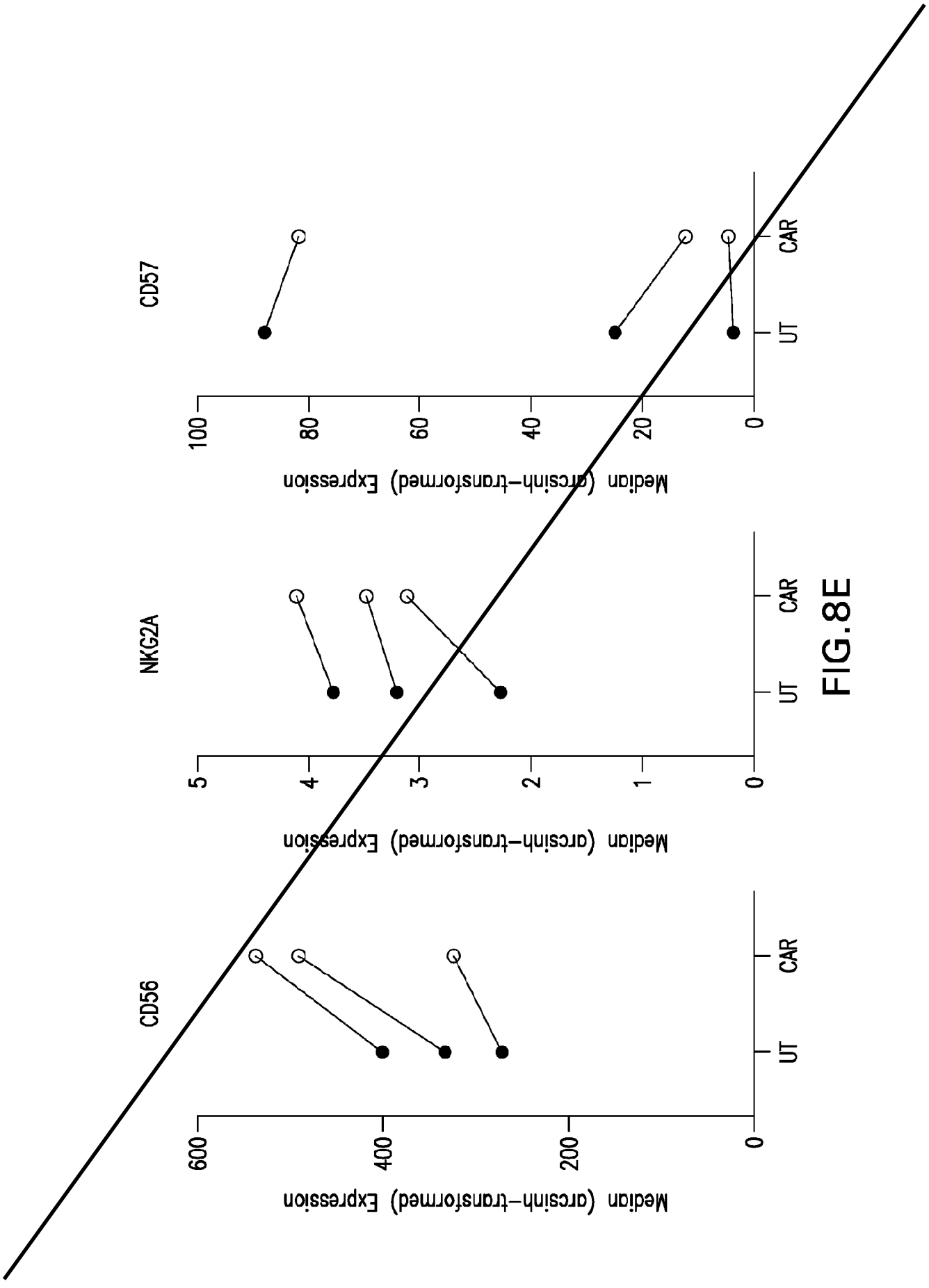

CD56
NKG2A
CD57
Median (arcsinh−transformed) Expression
0
200
400
600
1
2
3
4
5
20
40
60
80
100
UT
CAR

FIG.8E

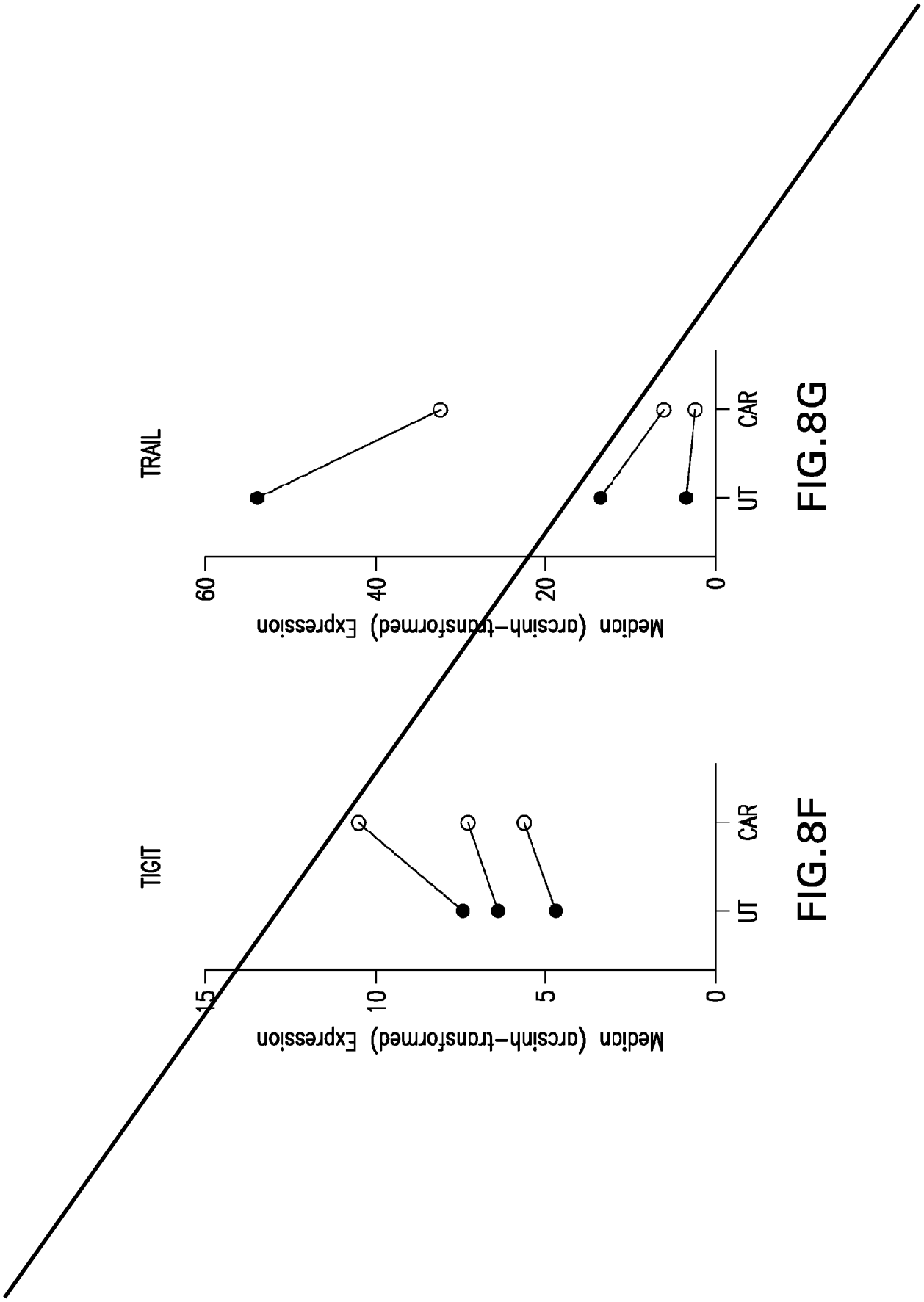

TIGIT
Median (arcsinh−transformed) Expression
0
5
10
15
UT
CAR
TRAIL
Median (arcsinh−transformed) Expression
0
20
40
60
UT
CAR

FIG.8F

FIG.8G

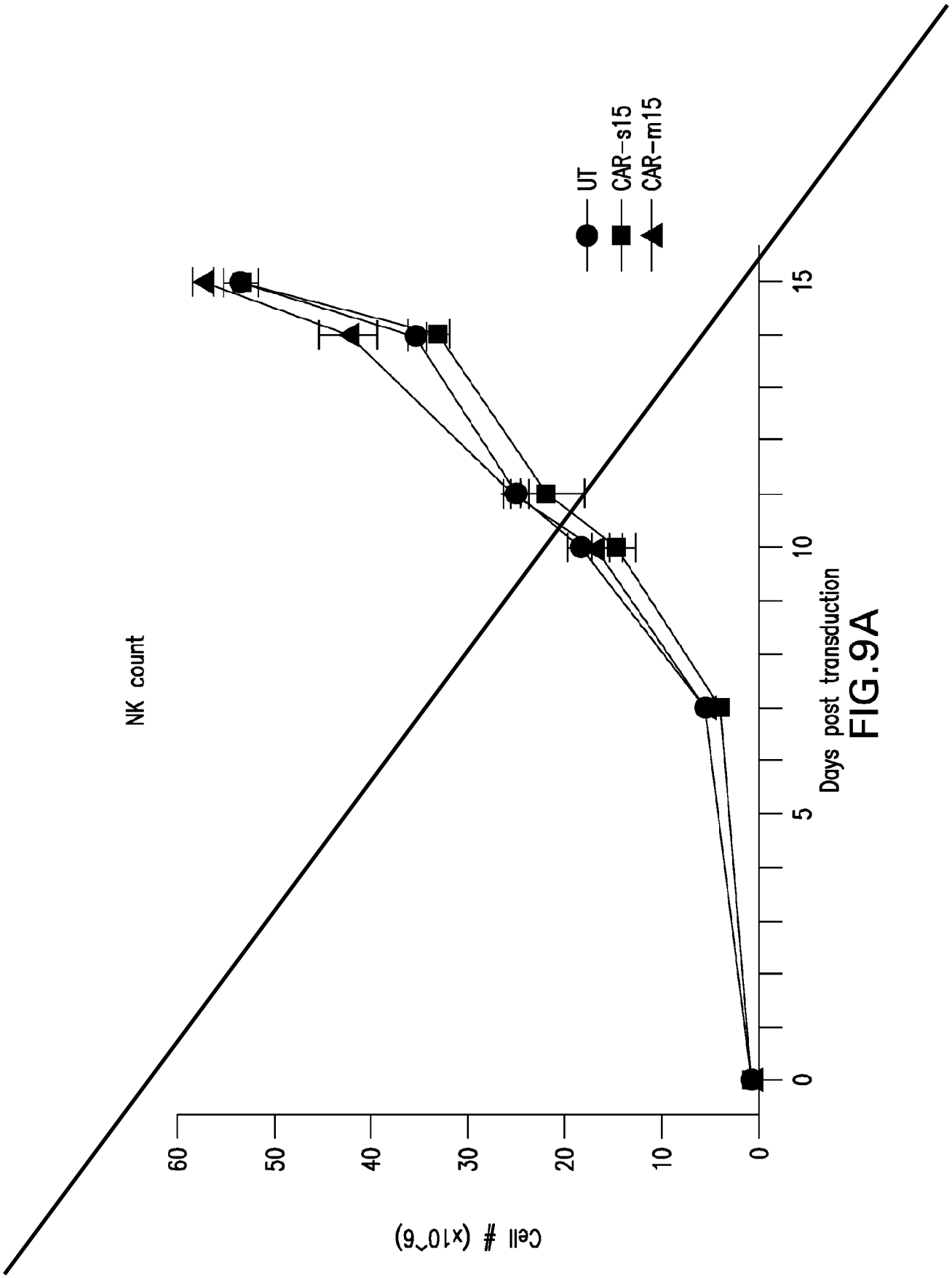

NK count
Cell # (x10^6)
60
50
40
30
20
10
0
0
5
10
15
Days post transduction
UT
CAR-s15
CAR-m15

FIG.9A

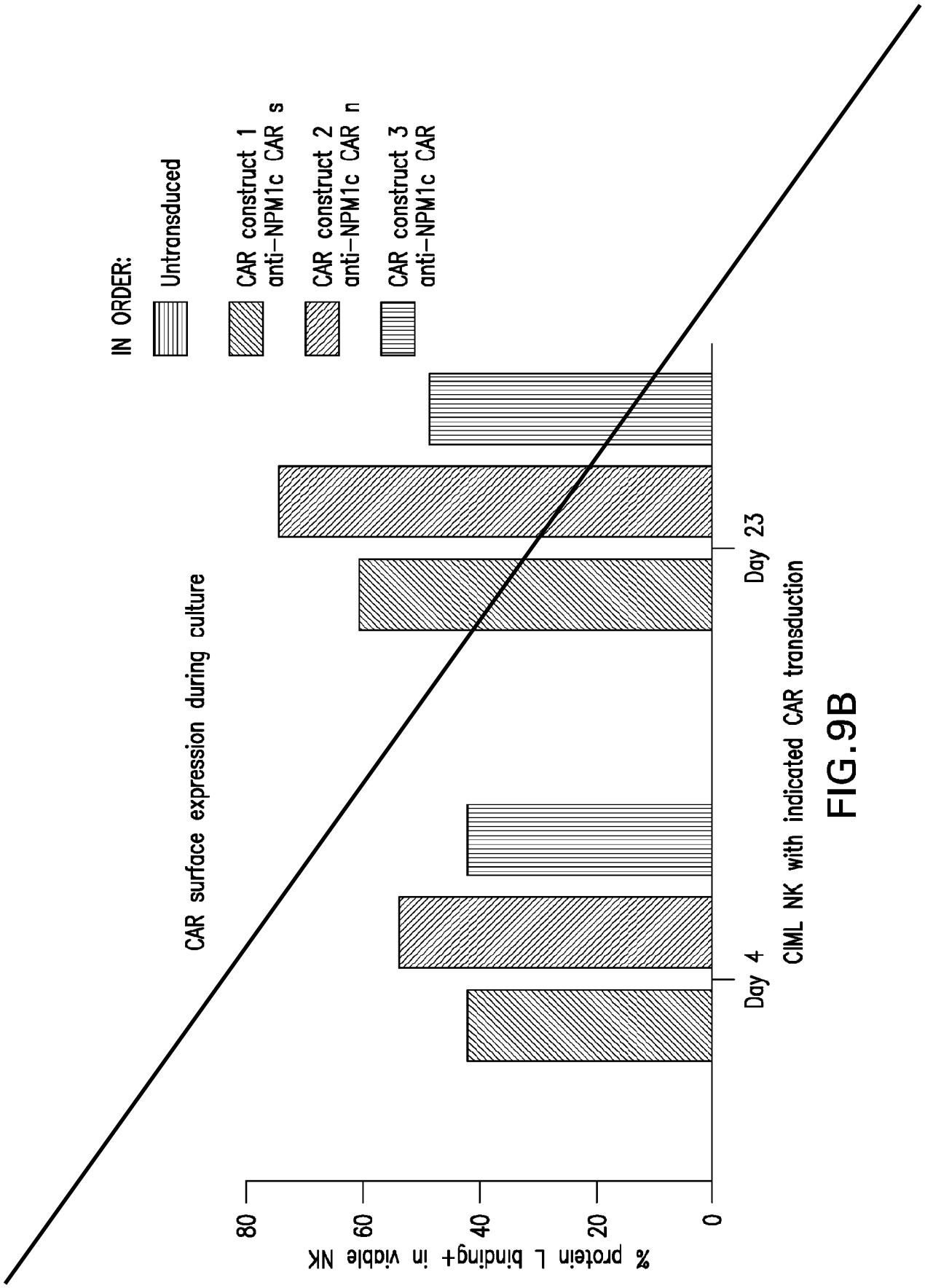

IN ORDER:
Untransduced
CAR construct 1 anti-NPM1c CAR s
CAR construct 2 anti-NPM1c CAR n
CAR construct 3 anti-NPM1c CAR
CAR surface expression during culture
% protein L binding+ in viable NK
0
20
40
60
80
Day 4
Day 23
CIML NK with indicated CAR transduction

FIG.9B

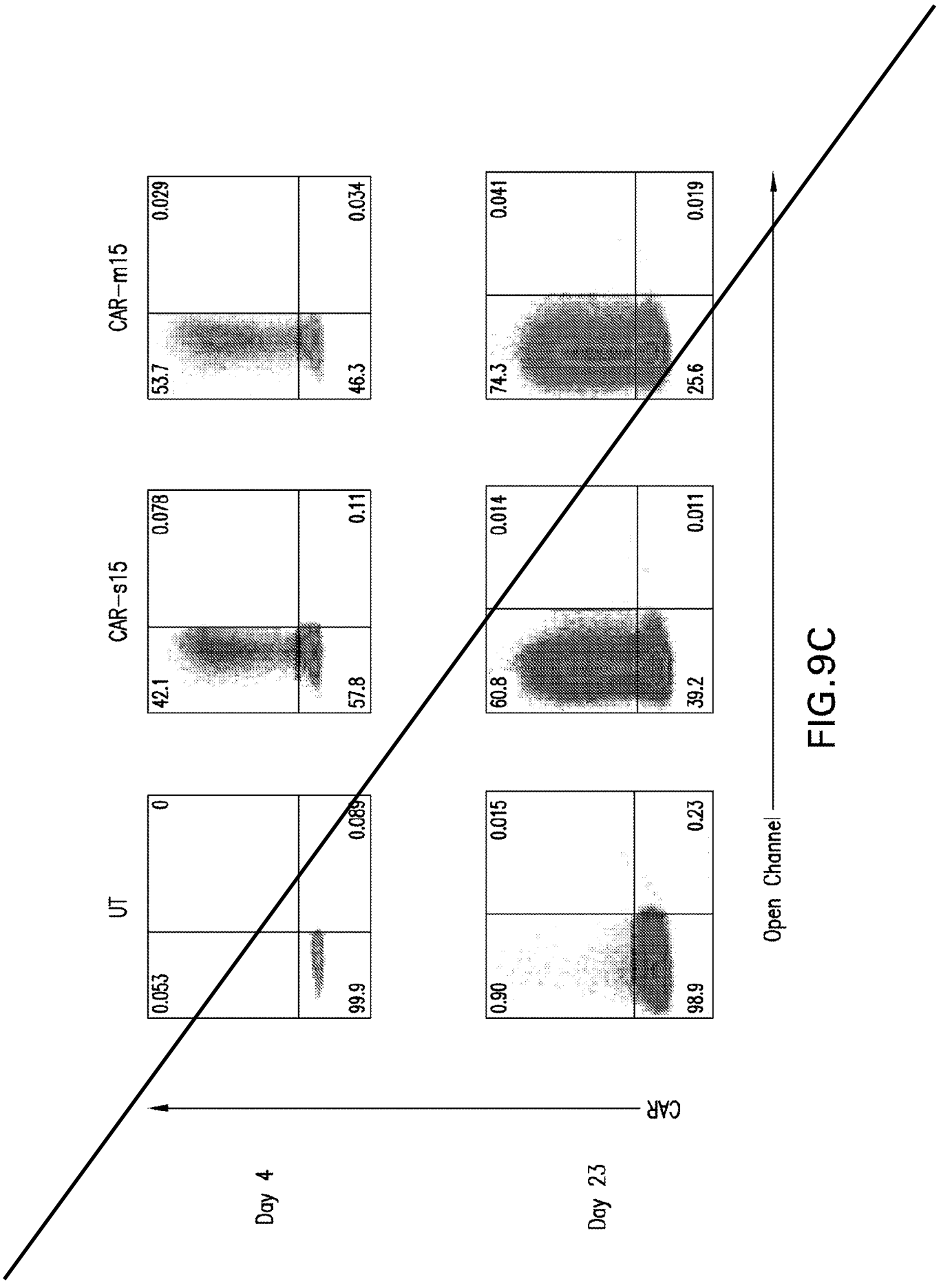

UT
CAR-s15
CAR-m15
0
0.089
0.053
99.9
0.078
0.11
42.1
57.8
0.029
0.034
53.7
46.3
0.015
0.23
0.90
98.9
0.014
0.011
60.8
39.2
0.041
0.019
74.3
25.6
Day 4
Day 23
CAR
Open Channel

FIG.9C

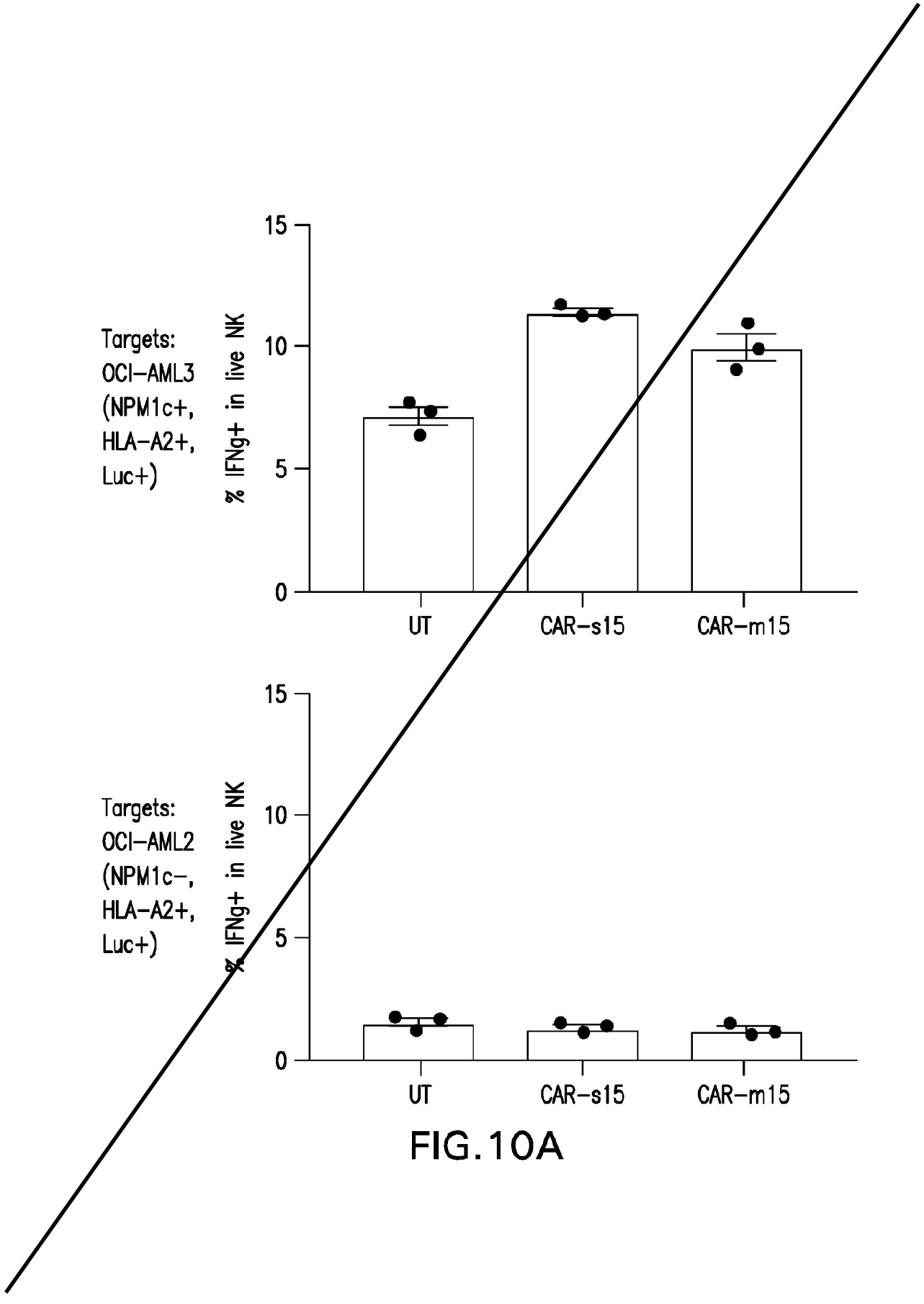
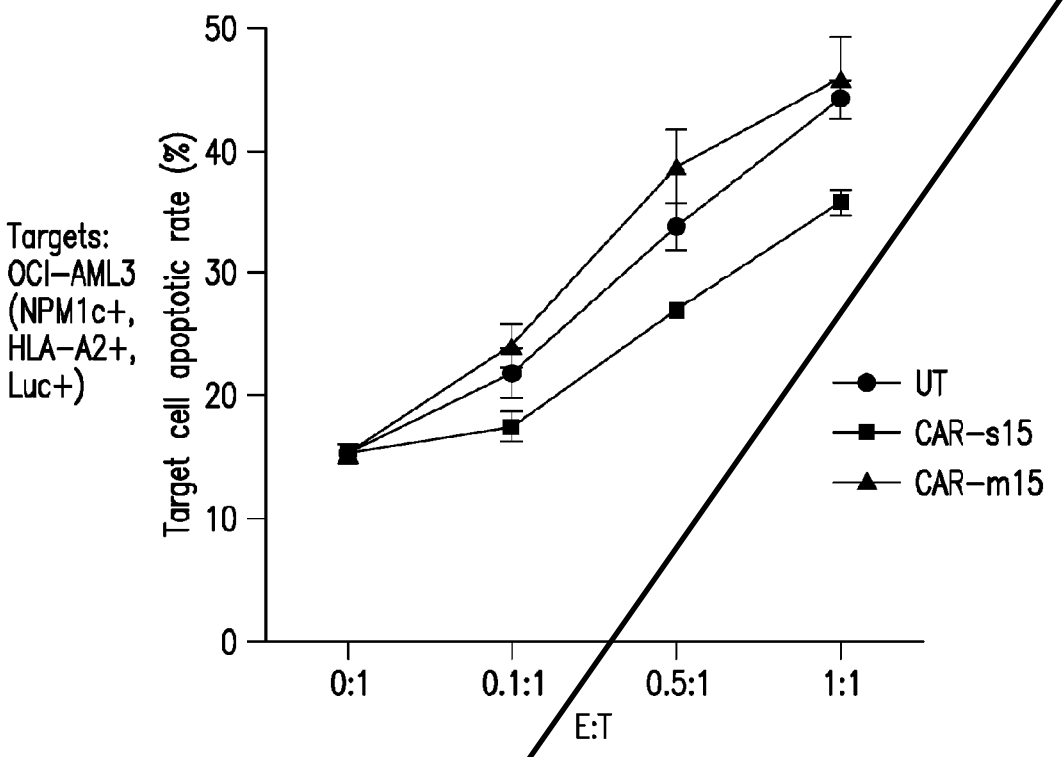

Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15
Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15

FIG.10A


Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR-s15
CAR-m15

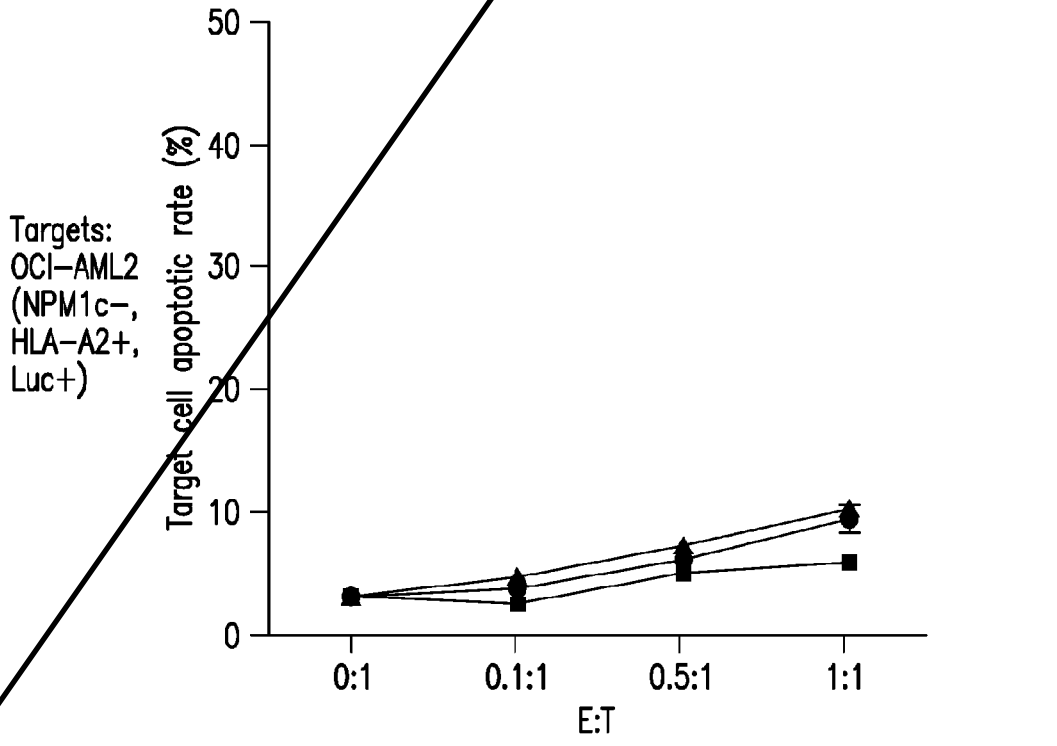

Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T

FIG.10B

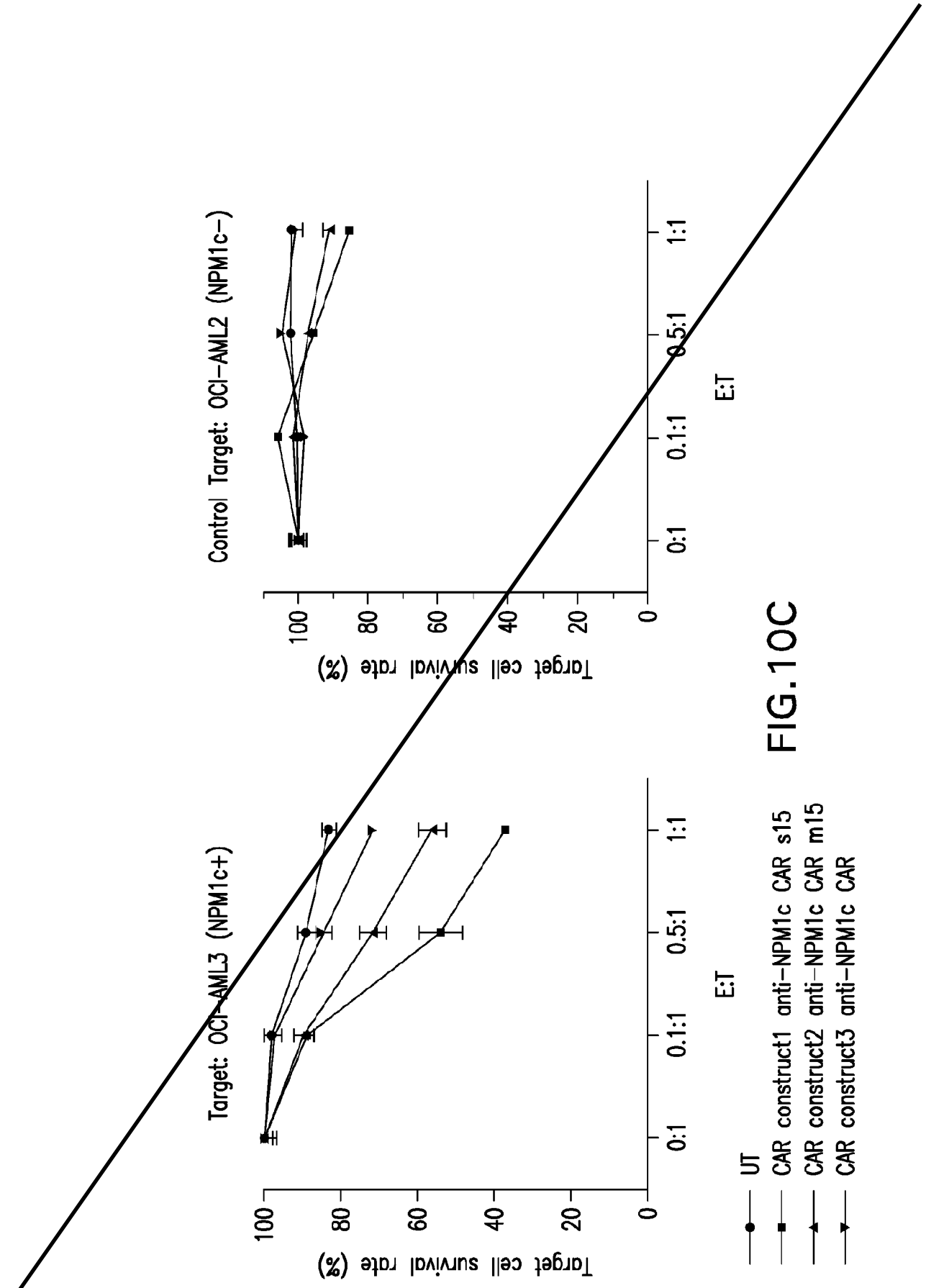

Target: OCI-AML3 (NPM1c+)
Control Target: OCI-AML2 (NPM1c-)
Target cell survival rate (%)
100
80
60
40
20
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR construct1 anti-NPM1c CAR s15
CAR construct2 anti-NPM1c CAR m15
CAR construct3 anti-NPM1c CAR

FIG.10C

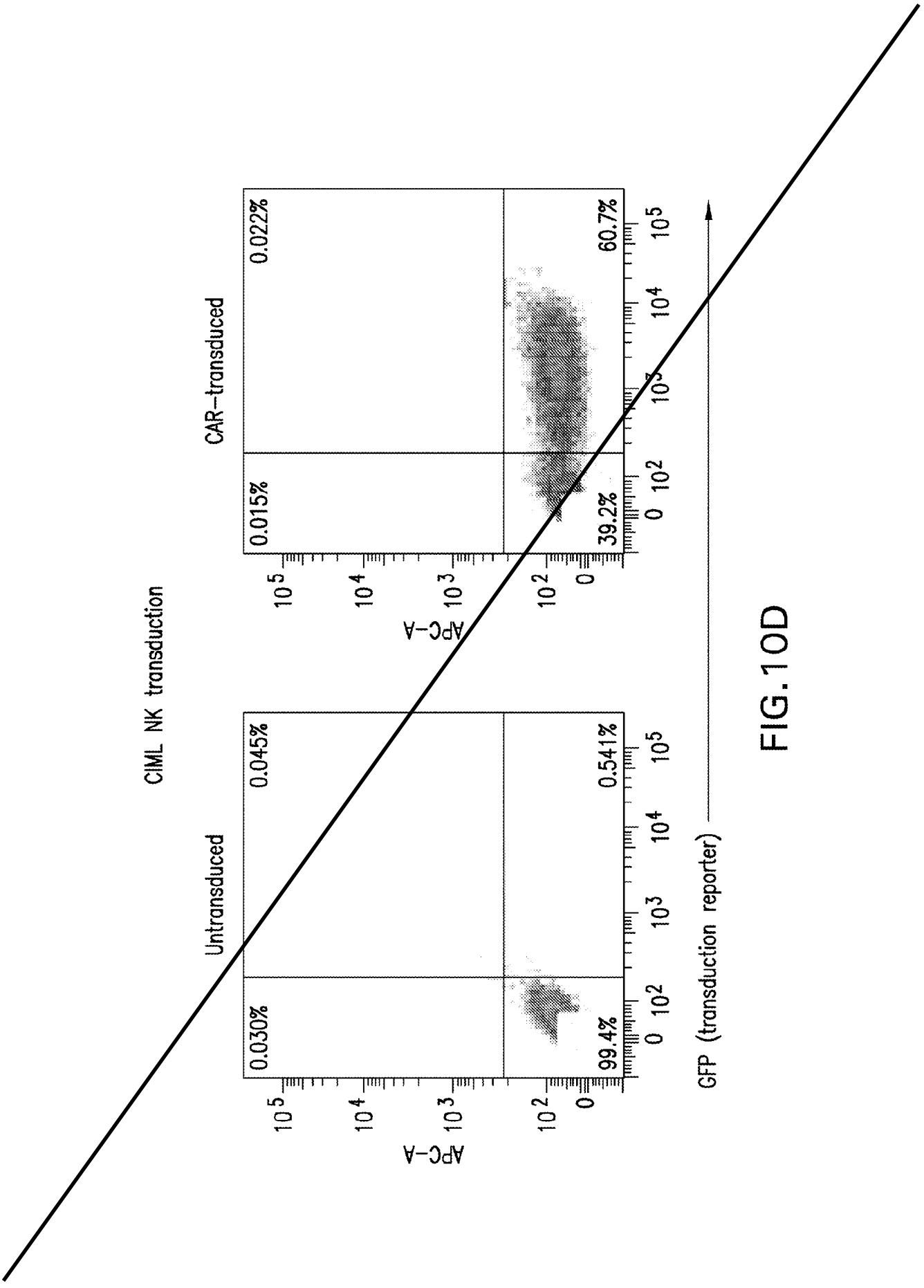

CIML NK transduction
Untransduced
CAR-transduced
APC-A
GFP (transduction reporter)
0.030%
0.045%
99.4%
0.541%
0.015%
0.022%
39.2%
60.7%

FIG.10D

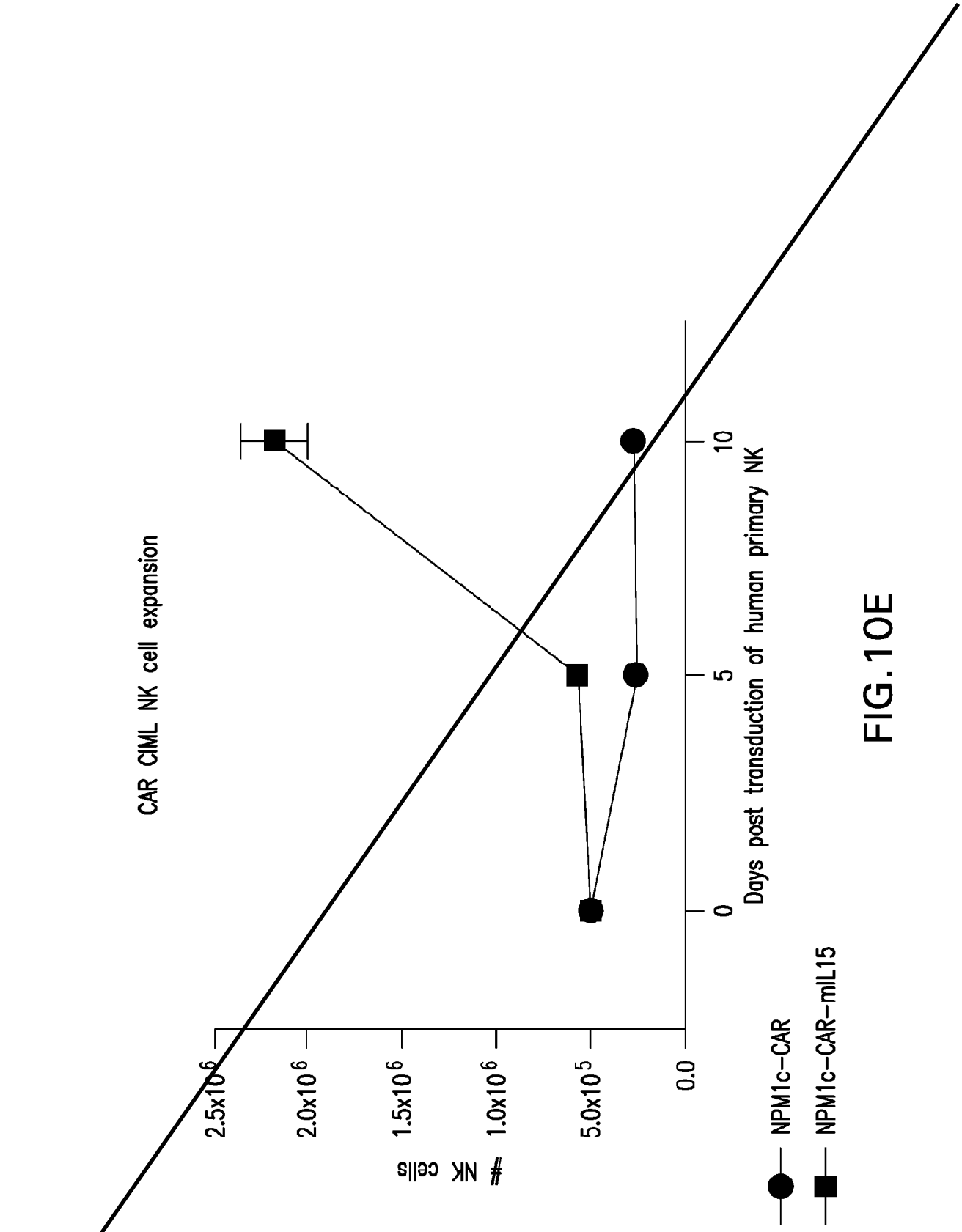

CAR CIML NK cell expansion
NK cells
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
5
10
Days post transduction of human primary NK
NPM1c-CAR
NPM1c-CAR-mIL15

FIG. 10E

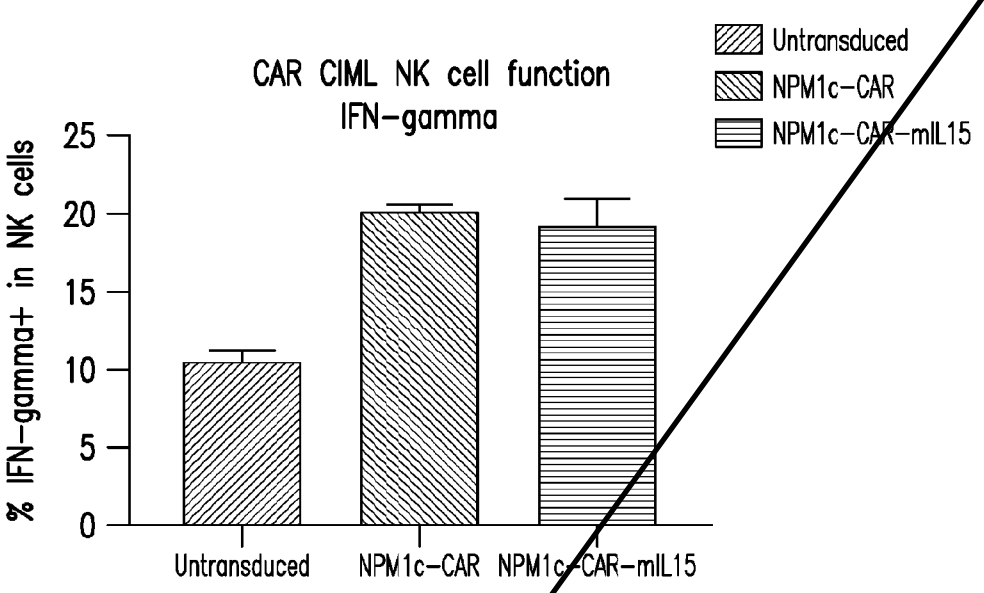


FIG.10F

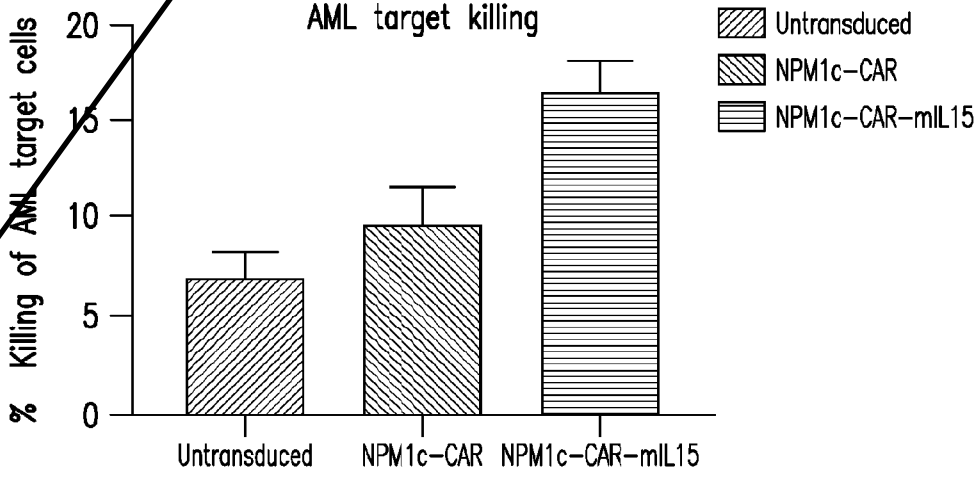


FIG.10G

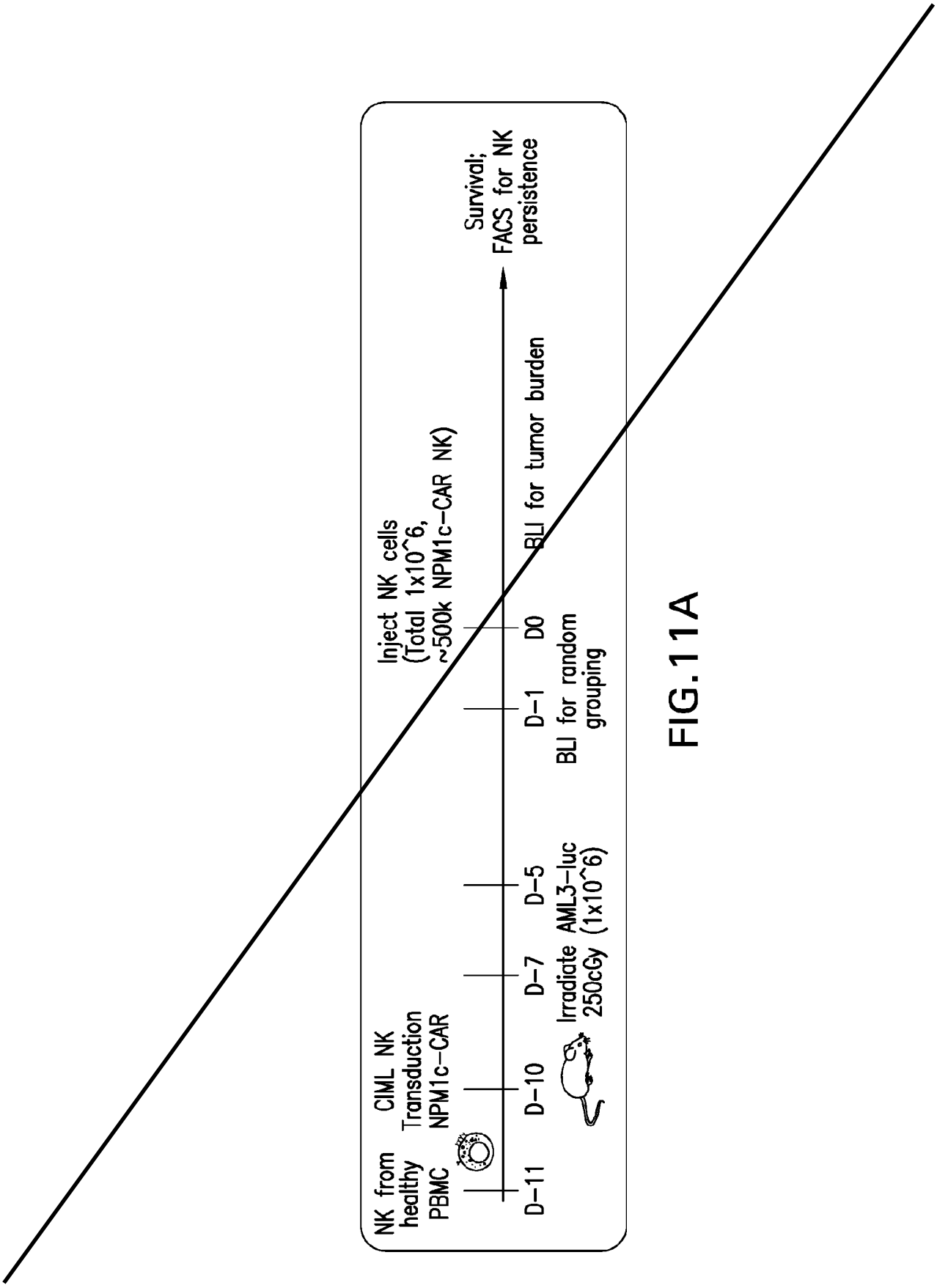

NK from healthy PBMC
CIML NK Transduction NPM1c-CAR
D-11
D-10
D-7
D-5
D-1
D0
Irradiate 250cGy
AML3-luc (1x10^6)
BLI for random grouping
Inject NK cells (Total 1x10^6, ~500k NPM1c-CAR NK)
BLI for tumor burden
Survival; FACS for NK persistence

FIG.11A

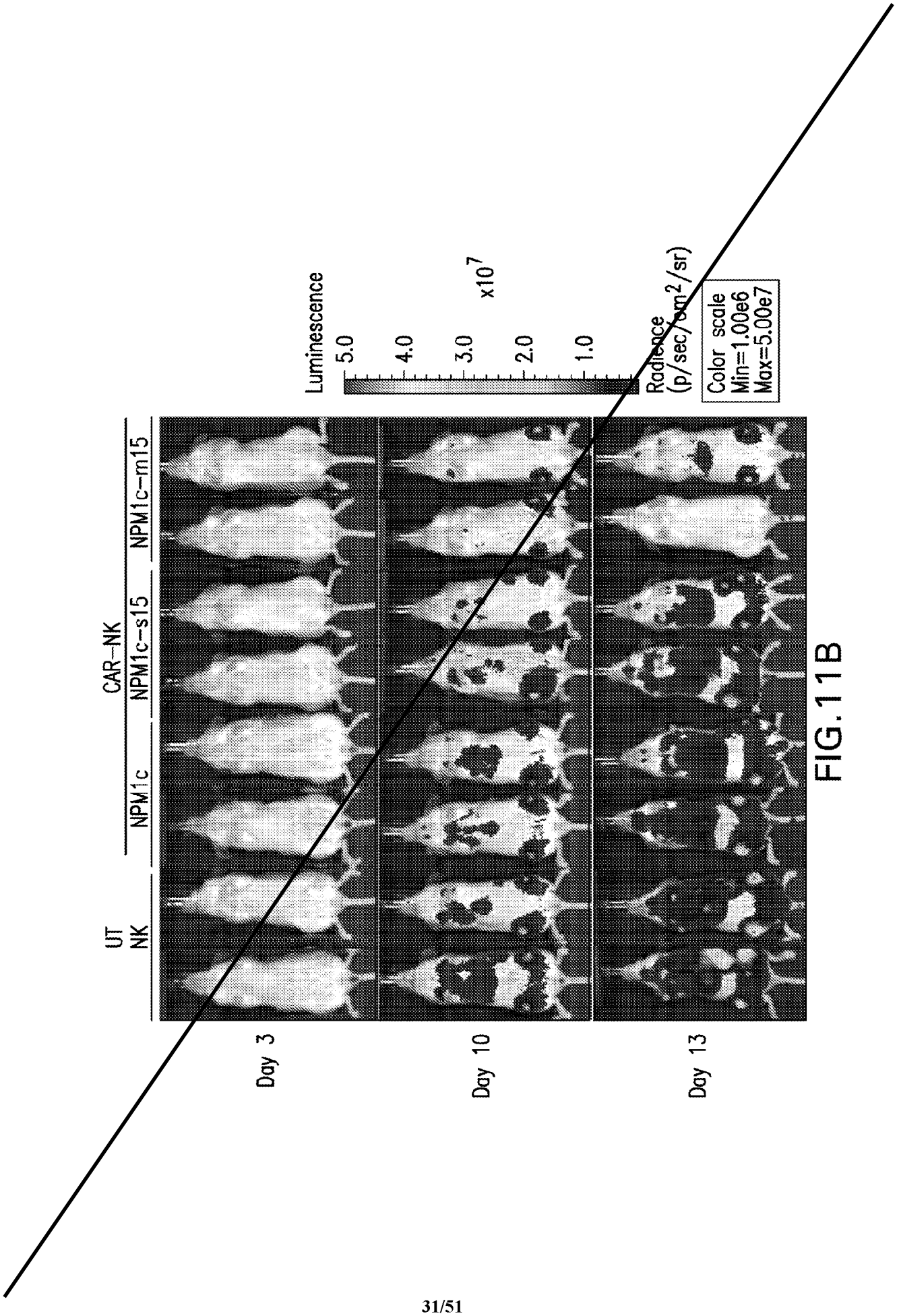

UT
NK
CAR-NK
NPM1c
NPM1c-s15
NPM1c-m15
Day 3
Day 10
Day 13
Luminescence
5.0
4.0
3.0
2.0
1.0
x10^7
Radiance (p/sec/cm^2/sr)
Color scale
Min=1.00e6
Max=5.00e7

FIG.11B

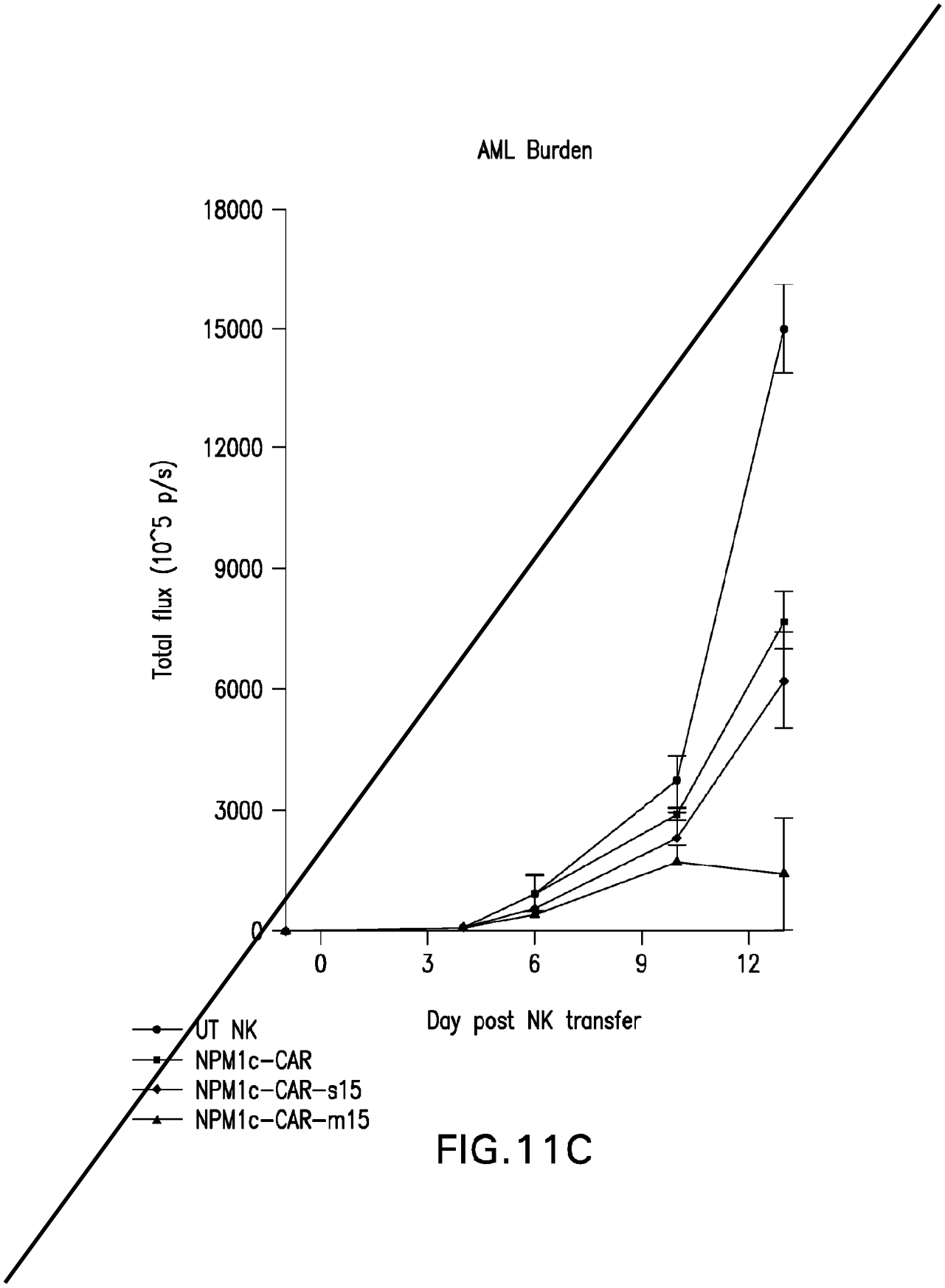

AML Burden
Total flux (10^5 p/s)
18000
15000
12000
9000
6000
3000
0
0
3
6
9
12
Day post NK transfer
UT NK
NPM1c-CAR
NPM1c-CAR-s15
NPM1c-CAR-m15

FIG.11C

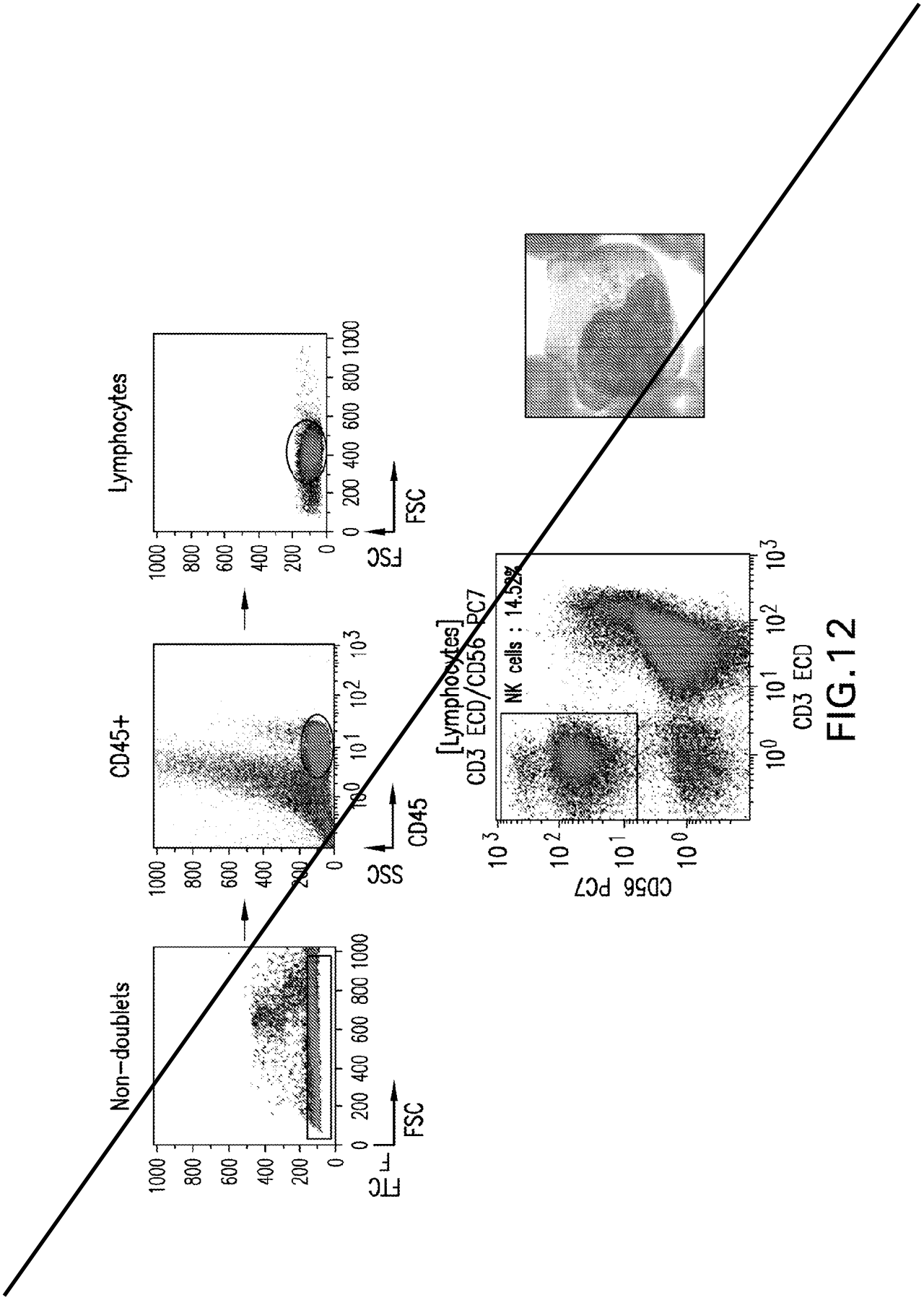

Non-doublets
FSC
SSC
CD45+
CD45
Lymphocytes
FSC
[Lymphocytes]
CD3 ECD/CD56 PC7
NK cells : 14.52%
CD56 PC7
CD3 ECD

FIG.12

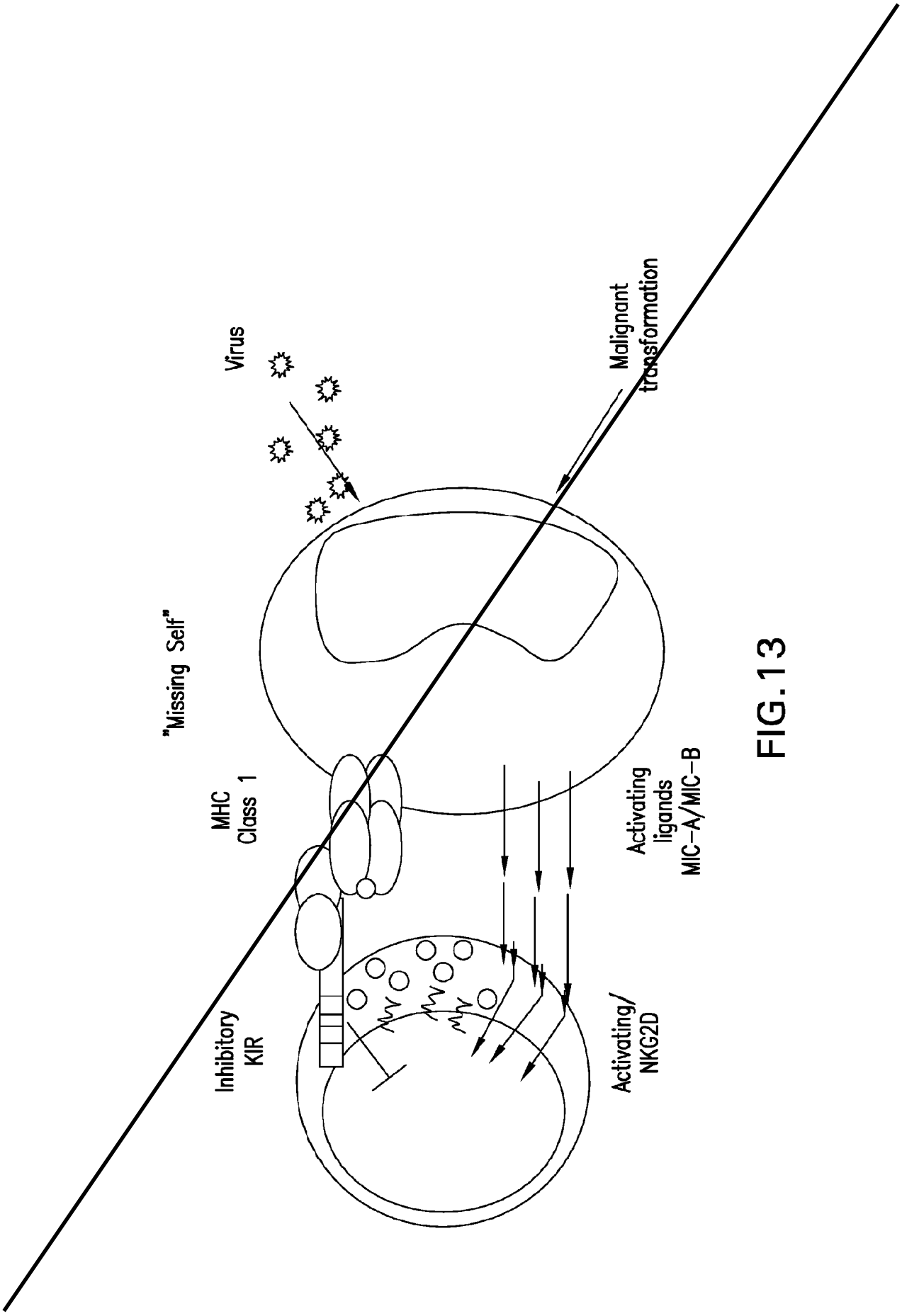

Virus
Malignant transformation
"Missing Self"
MHC Class 1
Activating ligands MIC-A/MIC-B
Inhibitory KIR
Activating/ NKG2D

FIG. 13

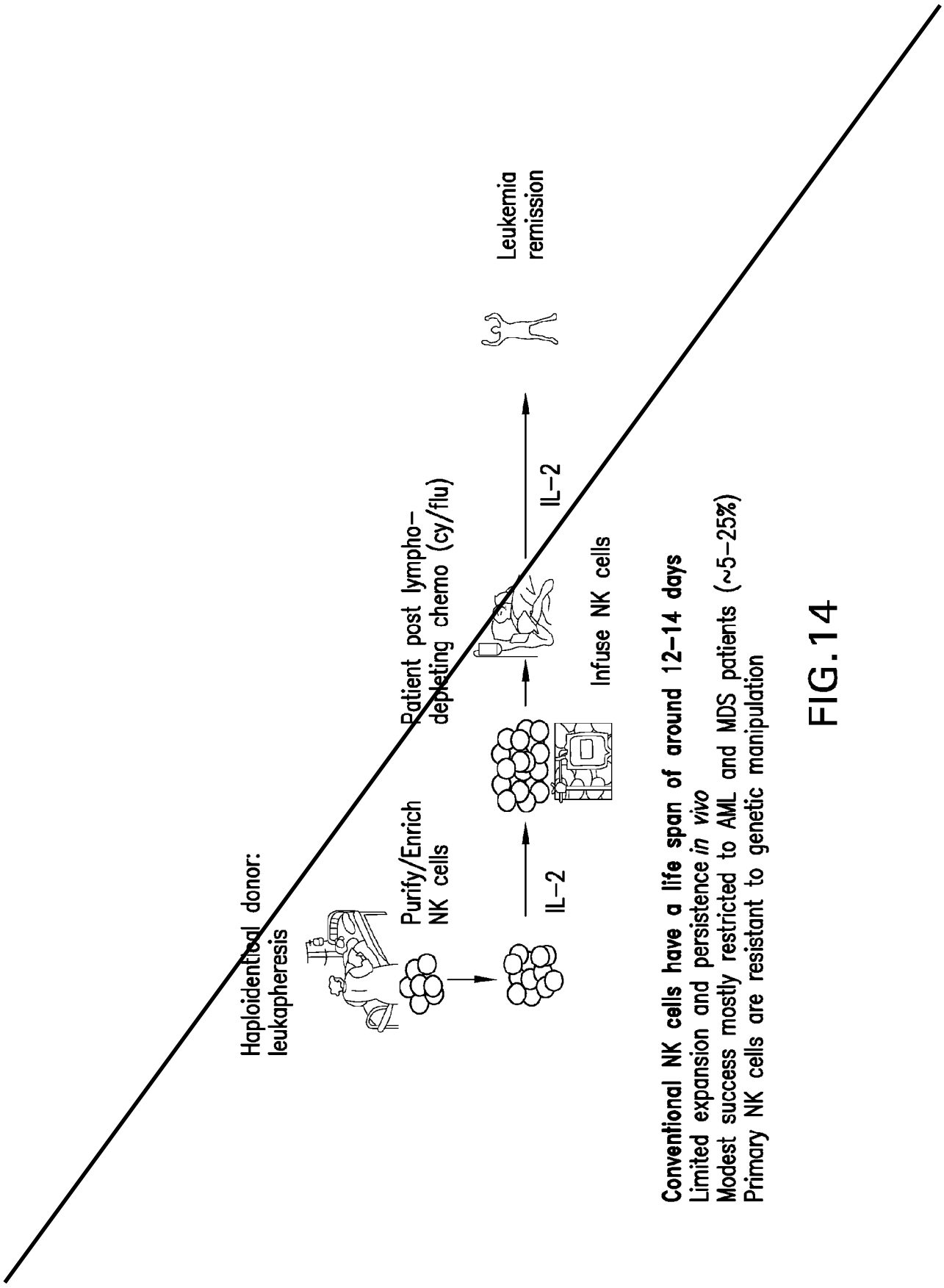

Haploidentical donor:
leukapheresis
Purify/Enrich
NK cells
IL-2
Patient post lympho-
depleting chemo (cy/flu)
IL-2
Infuse NK cells
Leukemia
remission
Conventional NK cells have a life span of around 12-14 days
Limited expansion and persistence in vivo
Modest success mostly restricted to AML and MDS patients (~5-25%)
Primary NK cells are resistant to genetic manipulation

FIG.14

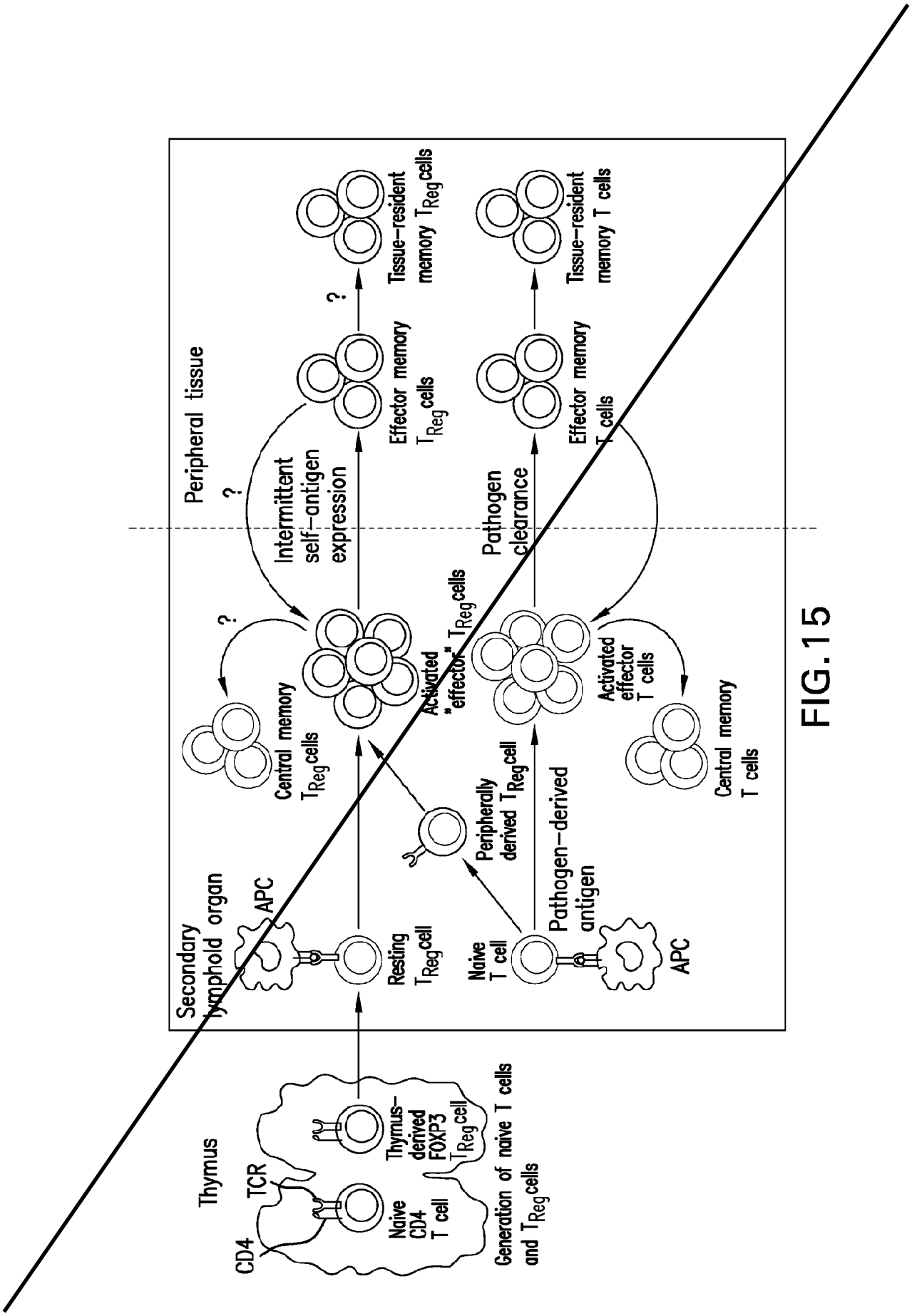

Thymus
CD4
TCR
Naive CD4 T cell
Thymus-derived FOXP3 T_{Reg} cell
Generation of naive T cells and T_{Reg} cells
Secondary lymphoid organ
APC
Resting T_{Reg} cell
Naive T cell
Pathogen-derived antigen
APC
Peripheral tissue
Central memory T_{Reg} cells
Peripherally derived T_{Reg} cell
Activated "effector" T_{Reg} cells
Intermittent self-antigen expression
Effector memory T_{Reg} cells
Tissue-resident memory T_{Reg} cells
Pathogen clearance
Activated effector T cells
Central memory T cells
Effector memory T cells
Tissue-resident memory T cells
?
?
?

FIG. 15

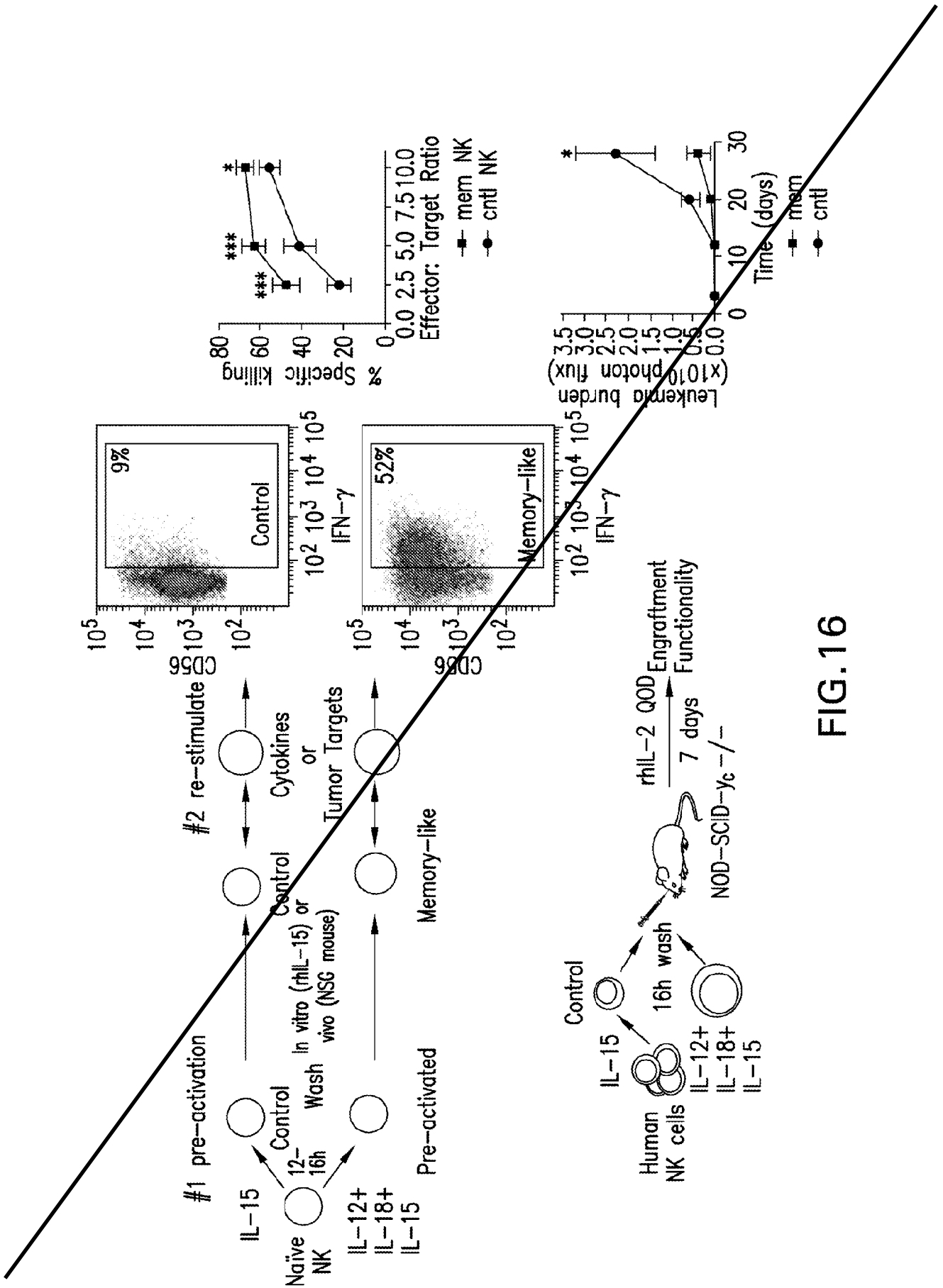

#1 pre-activation
Naïve NK
IL-15
IL-12+ IL-18+ IL-15
Control
12-16h
Wash
Pre-activated
Memory-like
In vitro (rhIL-15) or vivo (NSG mouse)
Control
#2 re-stimulate
Cytokines or Tumor Targets
CD56
IFN-γ
9%
Control
52%
Memory-like
% Specific killing
Effector: Target Ratio
mem NK
cntl NK
Leukemia burden (x10^10 photon flux)
Time (days)
mem
cntl
Human NK cells
IL-15
IL-12+ IL-18+ IL-15
16h wash
Control
NOD-SCID-γc -/-
rhIL-2 QOD
7 days
Engraftment Functionality

FIG.16

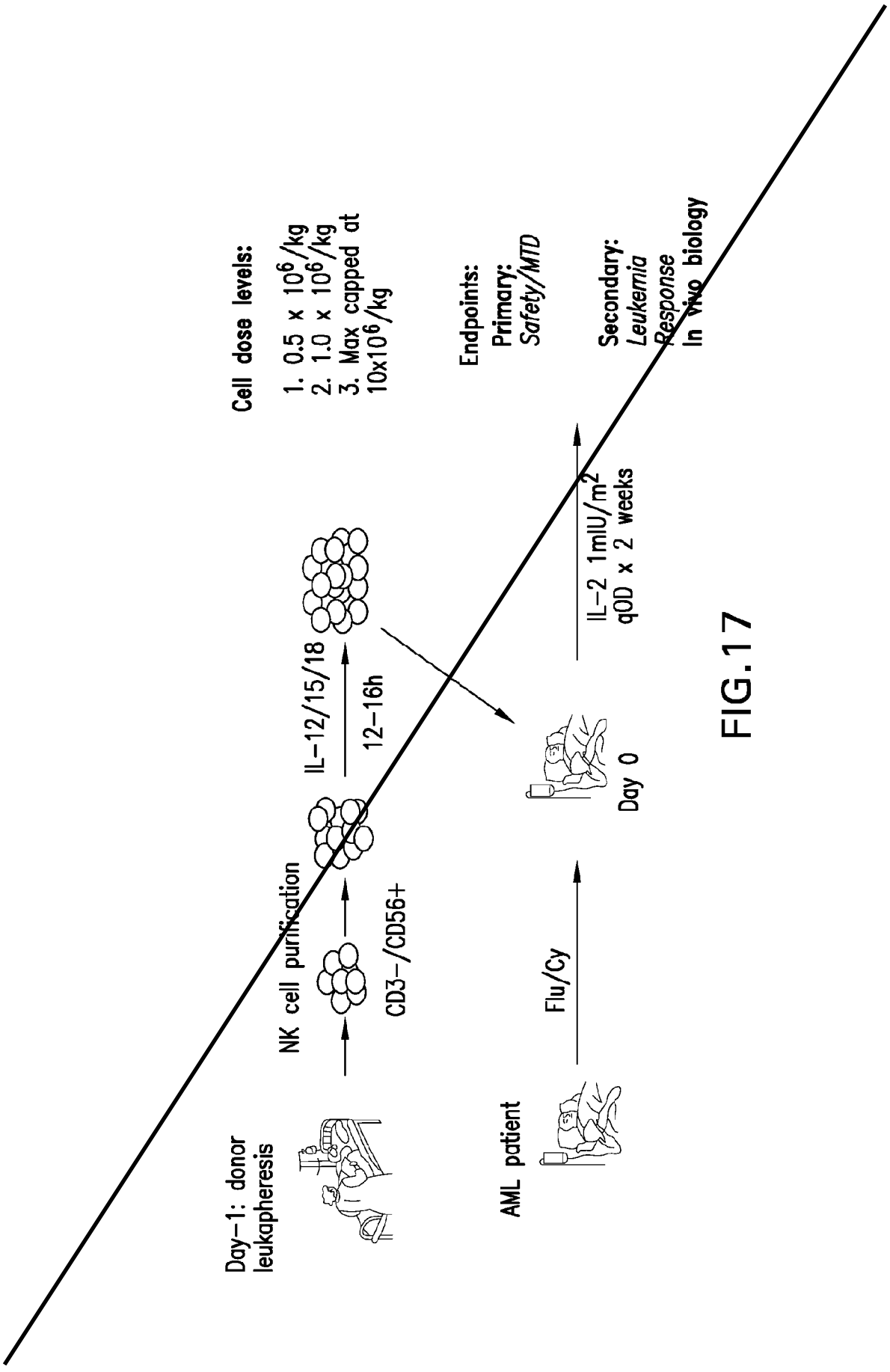

Day−1: donor leukapheresis
NK cell purification
CD3−/CD56+
IL−12/15/18
12−16h
AML patient
Flu/Cy
Day 0
IL−2 1mIU/m2
qOD x 2 weeks
Cell dose levels:
1. 0.5 x 10^6/kg
2. 1.0 x 10^6/kg
3. Max capped at 10x10^6/kg
Endpoints:
Primary:
Safety/MTD
Secondary:
Leukemia
Response
In vivo biology

FIG.17

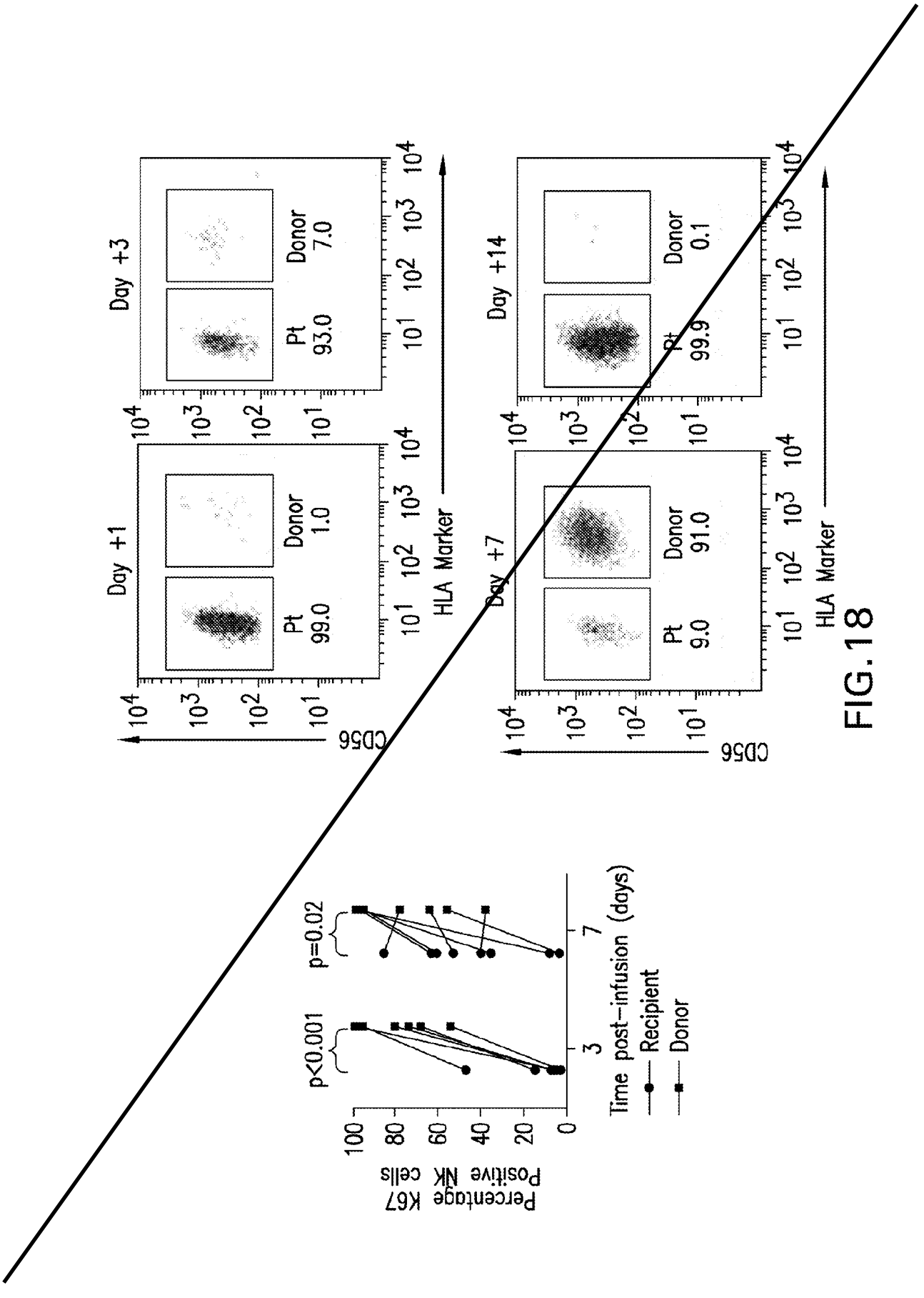

Day +1
Pt 99.0
Donor 1.0
Day +3
Pt 93.0
Donor 7.0
Day +7
Pt 9.0
Donor 91.0
Day +14
Pt 99.9
Donor 0.1
CD56
HLA Marker
p<0.001
p=0.02
Percentage K67 Positive NK cells
Time post-infusion (days)
Recipient
Donor

FIG.18

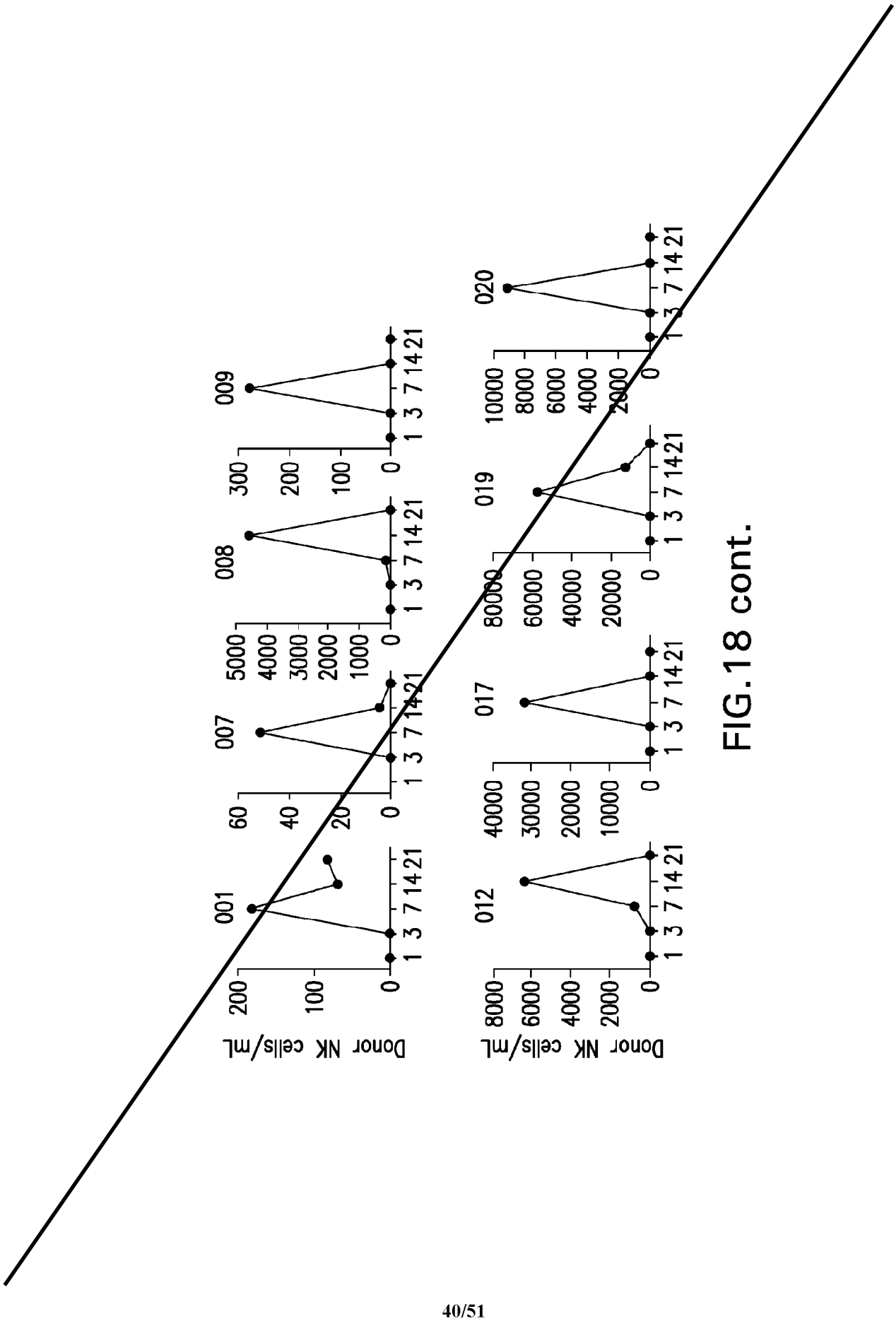


FIG.18 cont.

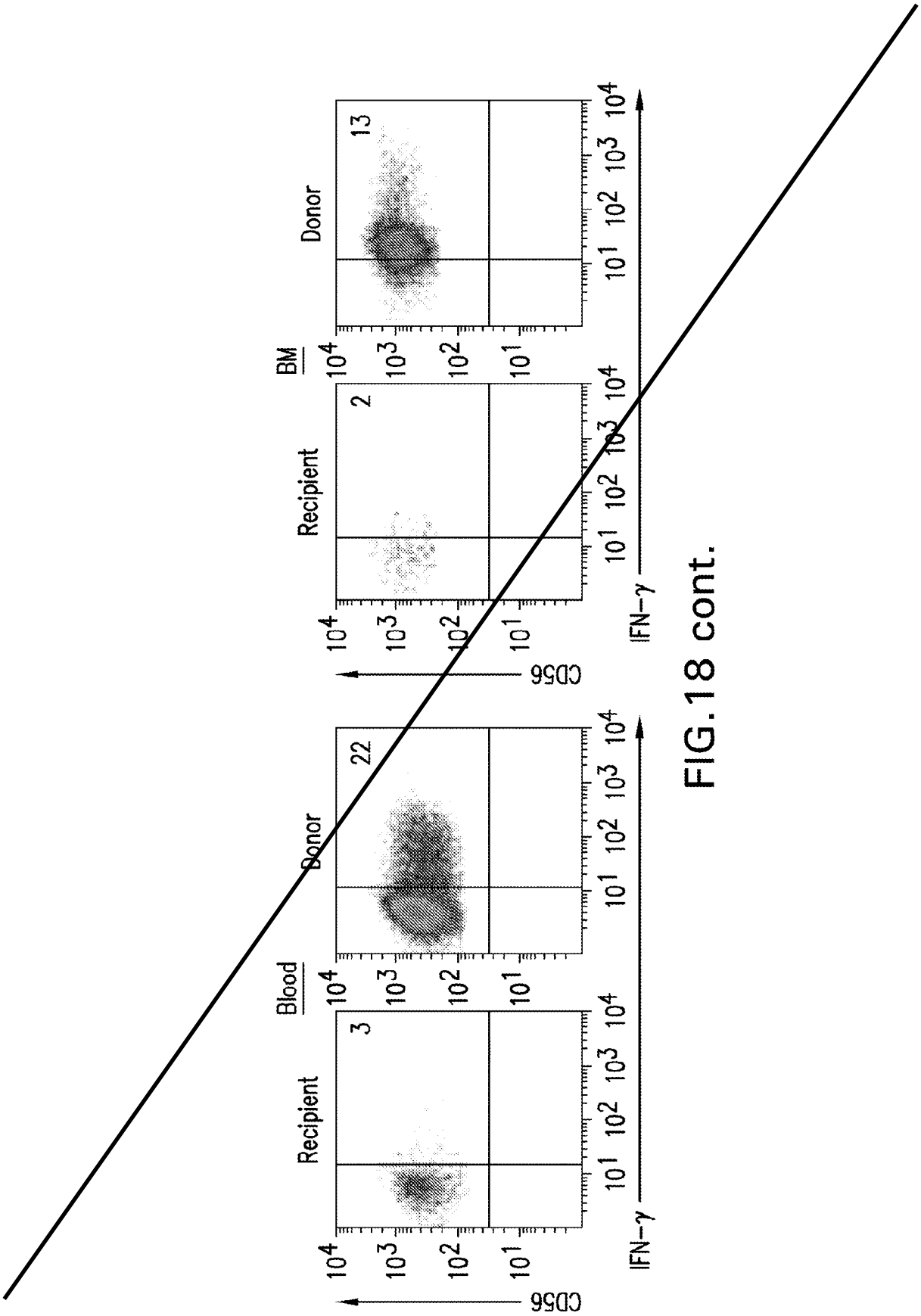

Blood
Recipient
3
Donor
22
BM
Recipient
2
Donor
13
CD56
IFN-γ
10^1 10^2 10^3 10^4

FIG. 18 cont.

UPN	Dose Level	Gender	Age (years)	WHO Dx	BM Blast (%)	KIR Ligand Mismatch	IWG Response	DLT	GVHD
001	1	M	73	M2	16	Yes	TF-PD	No	No
006	1	M	70	M0	28	Yes	TF-PD	No	No
007	1	M	77	M0	47	Yes	CR	No	No
008	2	M	76	t-AML	17	Yes	TF-PD	No	No
009	2	F	73	M1	80	No	MLFS	No	No
012	2	F	71	M5	15	Yes	CR	No	No
017	3	M	64	t-AML	69	Yes	TF-PD	No	No
019	3	F	71	M5	15	Yes	CR	No	No
020	3	M	60	MDS-AML	13	Yes	CRp	No	No
023	3	M	83	M1	86	No	CRp	No	No
025	3	M	72	t-AML	1	No	CR	No	No
027	3	M	76	M1	In CR	No	CRp	No	No
028	3	F	69	HMA failed MDS	6	No	Marrow CR	No	No

>50% CR Rat

2 Long term survivors

1 Died in CR

FIG.19

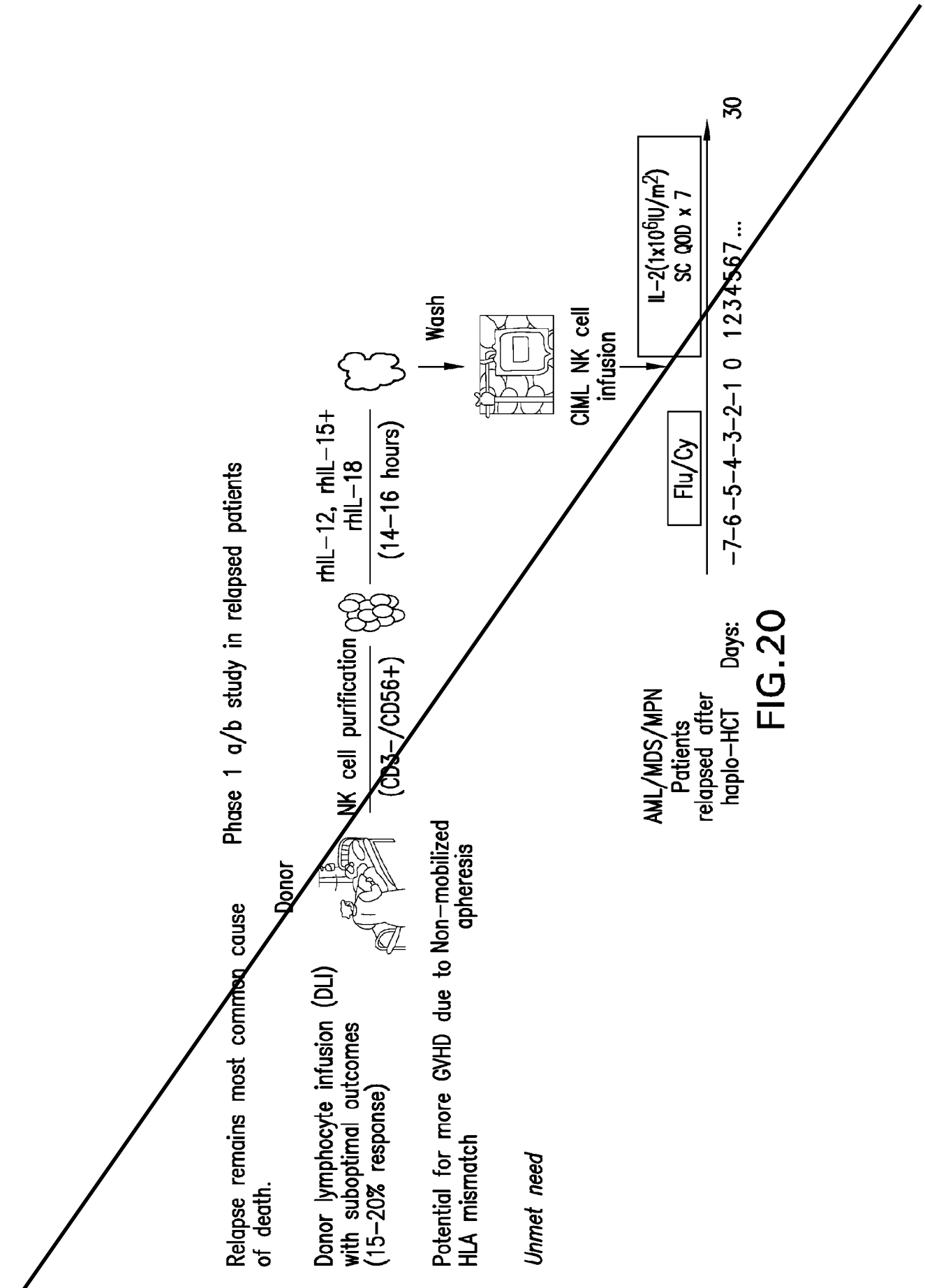

Relapse remains most common cause of death.
Phase 1 a/b study in relapsed patients
Donor
Donor lymphocyte infusion (DLI) with suboptimal outcomes (15–20% response)
NK cell purification
(CD3–/CD56+)
rhIL–12, rhIL–15+ rhIL–18
(14–16 hours)
Wash
CIML NK cell infusion
Potential for more GVHD due to Non–mobilized apheresis HLA mismatch
Unmet need
AML/MDS/MPN Patients relapsed after haplo–HCT
Flu/Cy
IL–2(1x10^6IU/m^2) SC QOD x 7
Days: –7–6–5–4–3–2–1 0 1 2 3 4 5 6 7 ... 30

FIG.20

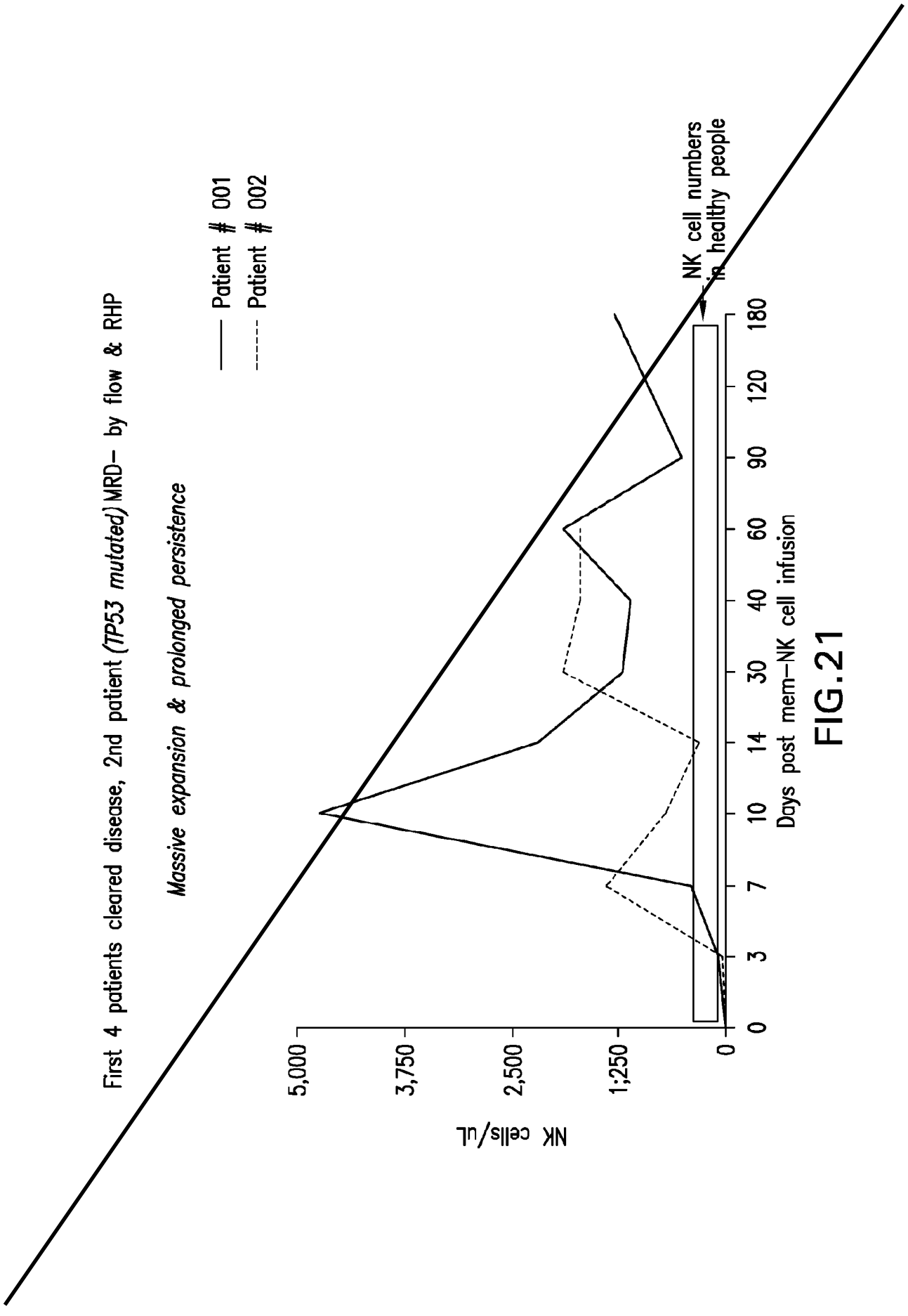

First 4 patients cleared disease, 2nd patient (TP53 mutated) MRD– by flow & RHP
Massive expansion & prolonged persistence
Patient # 001
Patient # 002
NK cell numbers in healthy people
NK cells/uL
5,000
3,750
2,500
1;250
0
0
3
7
10
14
30
40
60
90
120
180
Days post mem–NK cell infusion

FIG.21

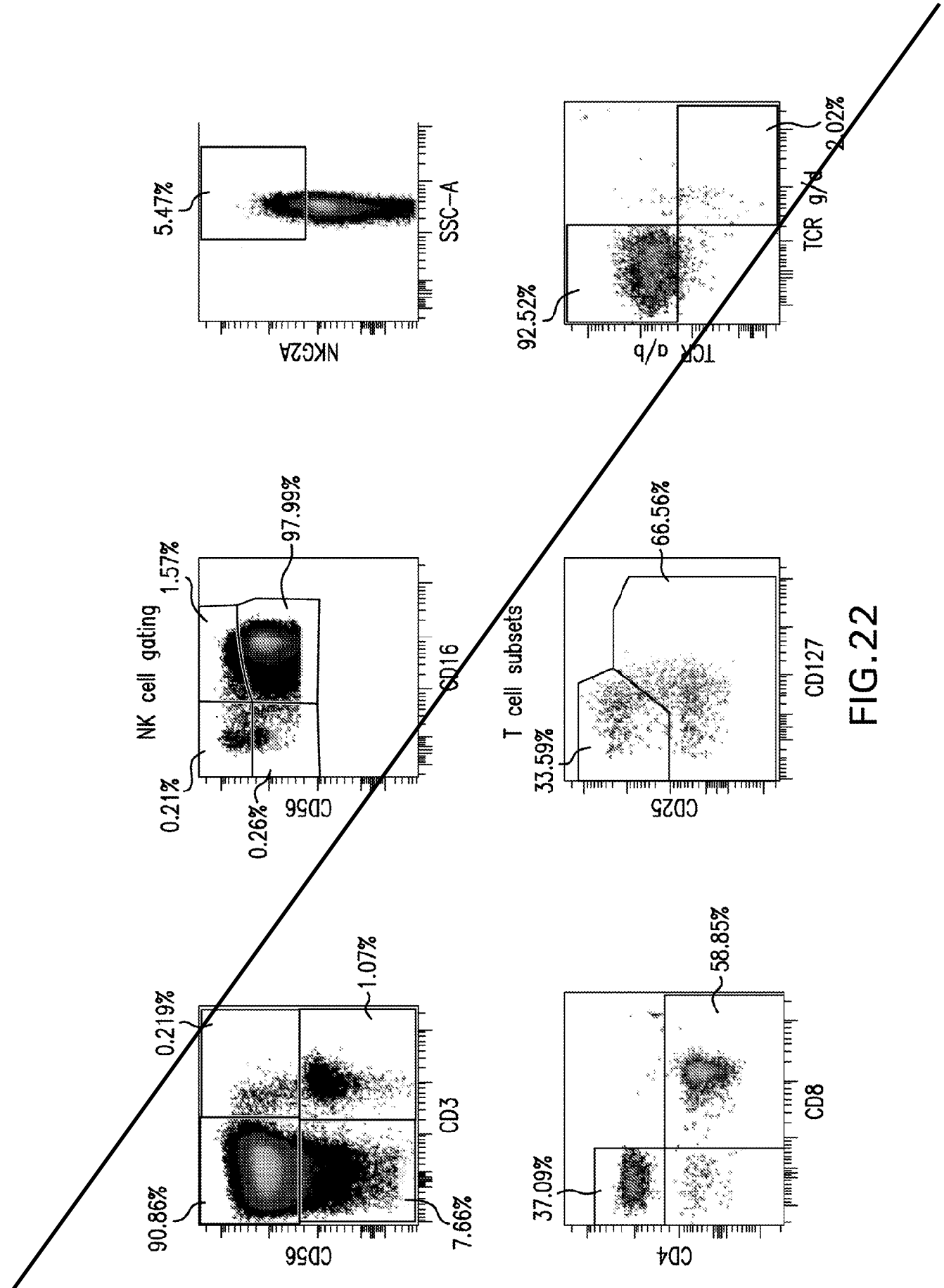

5.47%
SSC-A
NKG2A
92.52%
TCR g/d
TCR a/b
2.02%
NK cell gating
1.57%
97.99%
0.21%
0.26%
CD56
CD16
T cell subsets
66.56%
33.59%
CD25
CD127
0.219%
1.07%
90.86%
7.66%
CD56
CD3
58.85%
37.09%
CD4
CD8

FIG.22

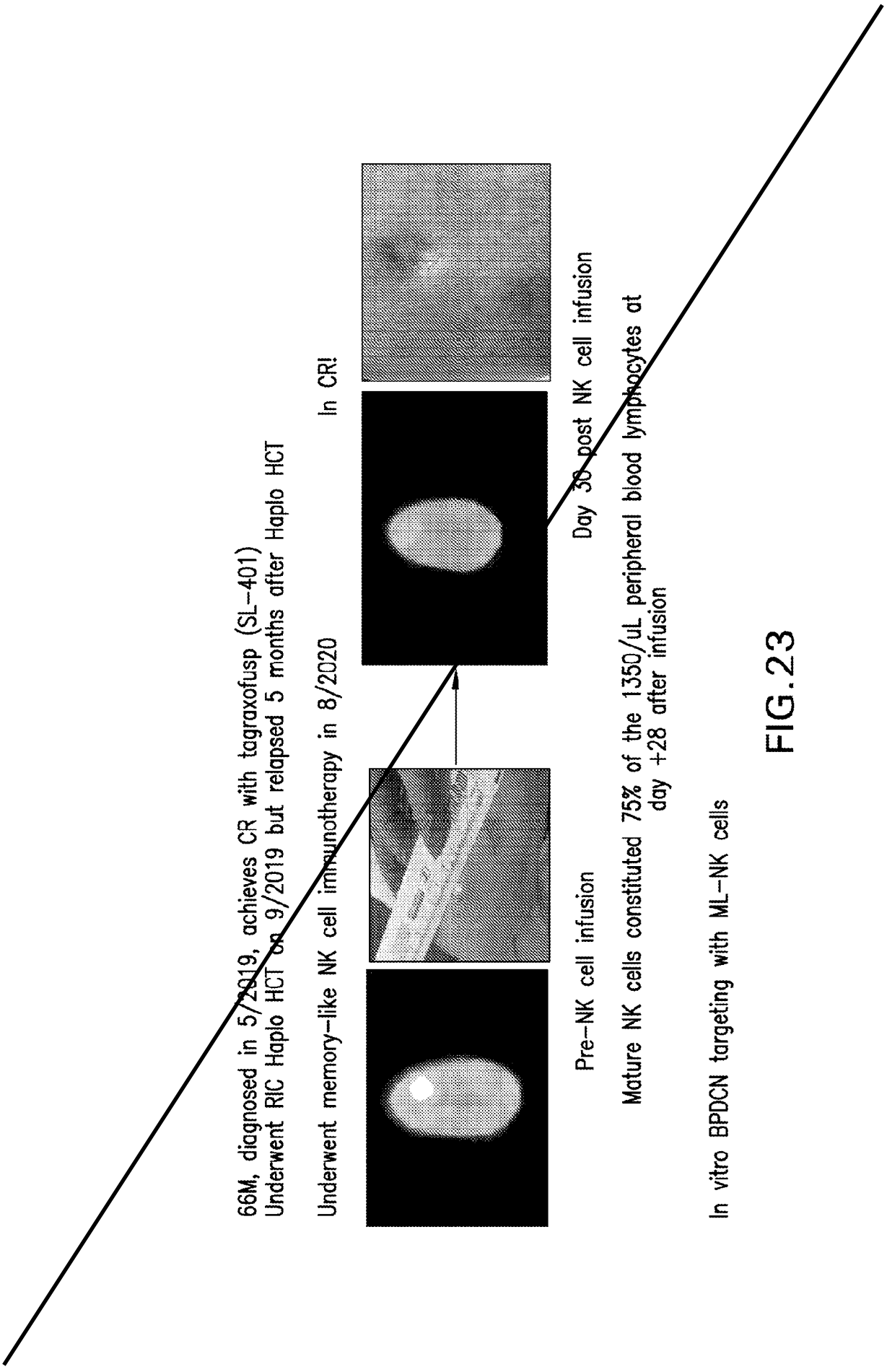

66M, diagnosed in 5/2019, achieves CR with tagraxofusp (SL-401)
Underwent RIC Haplo HCT on 9/2019 but relapsed 5 months after Haplo HCT
Underwent memory-like NK cell immunotherapy in 8/2020
In CR!
Pre-NK cell infusion
Day 30 post NK cell infusion
Mature NK cells constituted 75% of the 1350/uL peripheral blood lymphocytes at day +28 after infusion
In vitro BPDCN targeting with ML-NK cells

FIG.23

Anti-CTLA-4 antibody leads to Treg depletion to facilitate NK cell activity
NK cell infiltration associated with favorable outcomes in H&N ca patients
N-803 is an IL-15 super-agonist which enhances NK cell activation in vivo
Key Eligibility:
platinum-refractory disease
NK cell enrichment
CD3-/CD56+ selection
rhIL-12/15/18
CIML NK cell infusion
Ipilimumab (3mg/kg)
Flu/Cy
N-803 Q21 days
-21 -7-6-5-4-3-2-1 0 1 2 3 4 5 6 7 8 9 10 11... 30
CD56
NK cells 90.0
T cells 0.41
CD3
First patient treated on 9/15 (platinum/cetux/pembro failure)
>2500 mature NK cells/uL on day 21
IL-15 injection site rash

FIG.24

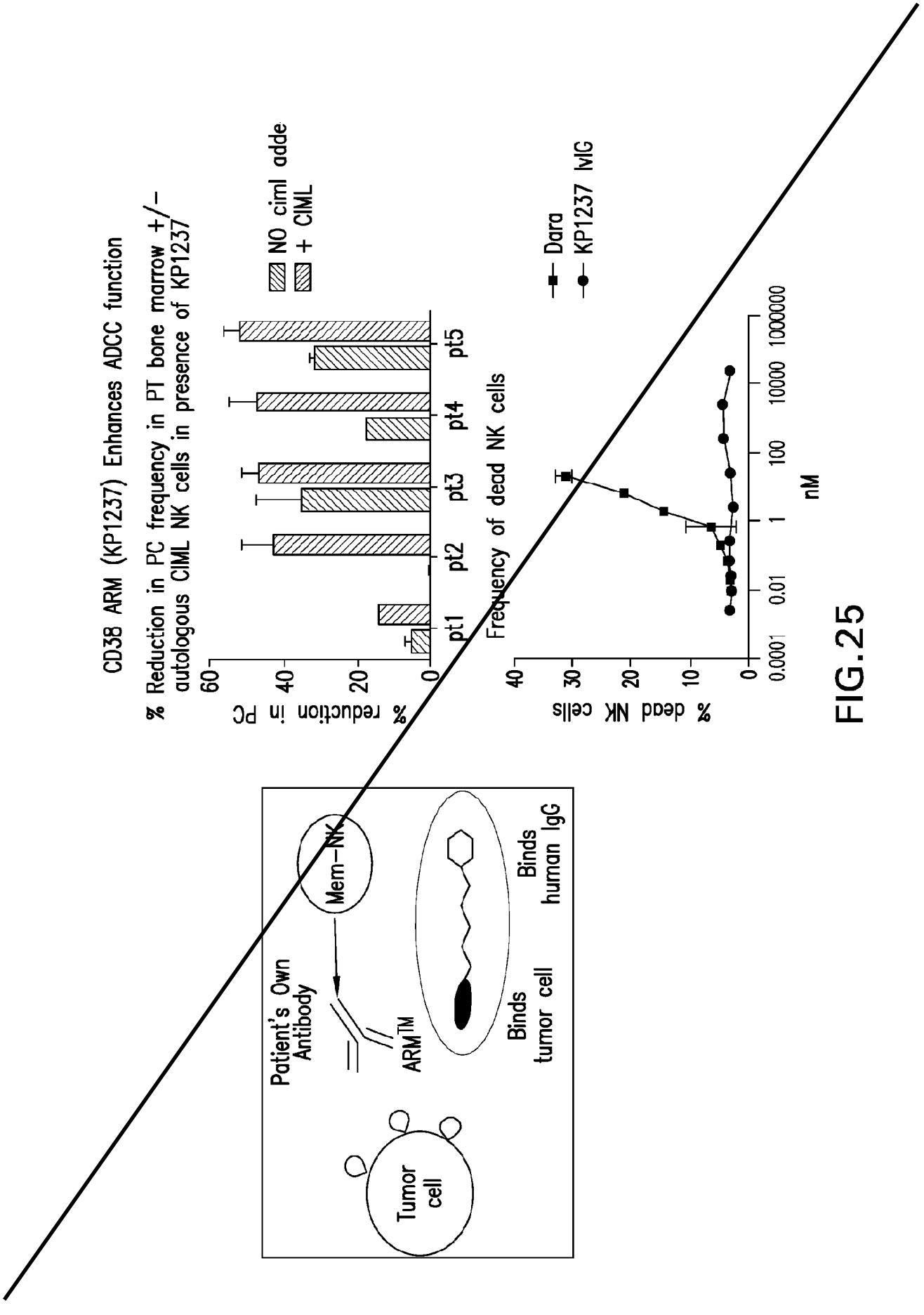

CD38 ARM (KP1237) Enhances ADCC function
% Reduction in PC frequency in PT bone marrow +/- autologous CIML NK cells in presence of KP1237
NO ciml adde
+ CIML
% reduction in PC
60
40
20
0
pt1
pt2
pt3
pt4
pt5
Frequency of dead NK cells
Dara
KP1237 IvIG
% dead NK cells
40
30
20
10
0
0.0001
0.01
1
100
10000
1000000
nM
Patient's Own Antibody
Mem-NK
ARM™
Binds tumor cell
Binds human IgG
Tumor cell

FIG.25

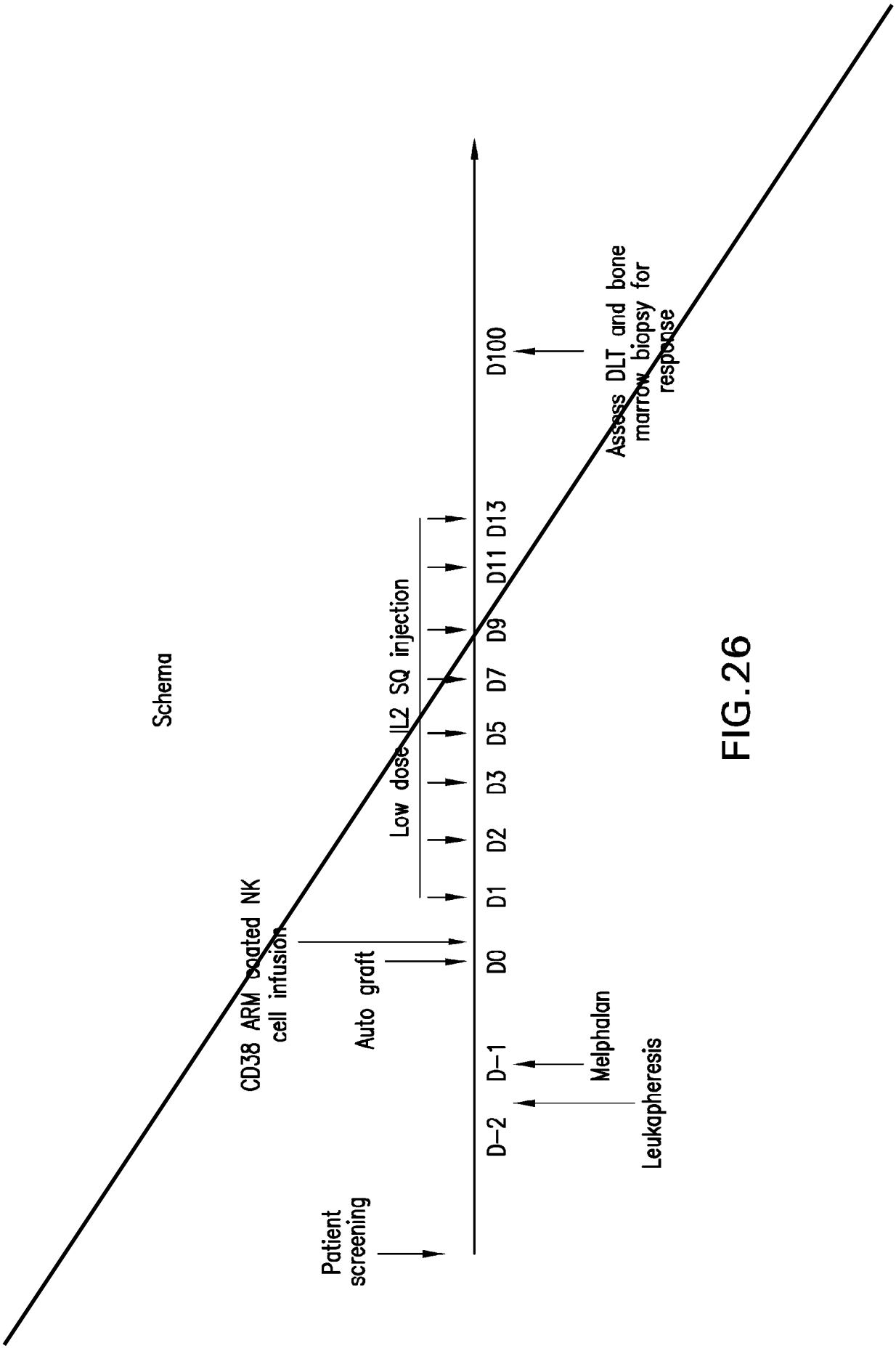

Schema
Patient screening
D-2
D-1
Melphalan
Leukapheresis
D0
Auto graft
CD38 ARM coated NK cell infusion
D1
D2
D3
D5
D7
D9
D11
D13
Low dose IL2 SQ injection
D100
Assess DLT and bone marrow biopsy for response

FIG.26

NK cell CARs are safe (no CRS/no neurotoxicity), response rate similar to CAR T cells
Limited efficacy of CAR T cells in AML/MDS
Dual targeting of myeloid blasts by Mem-NK cells armed with CARs (antigen expression triggers CAR vs HLA loss triggers direct NK cell cytotoxicity)
Primary Peripheral blood NK cells are resistant to transduction
72hr post-transduction
Lenti-NPM1c-CAR-GFP
Untransduced
IL-12
IL-12+18
PB-NK cells
APC
GFP
% transduction
0.78
0.77
5.59
43.36

FIG.27

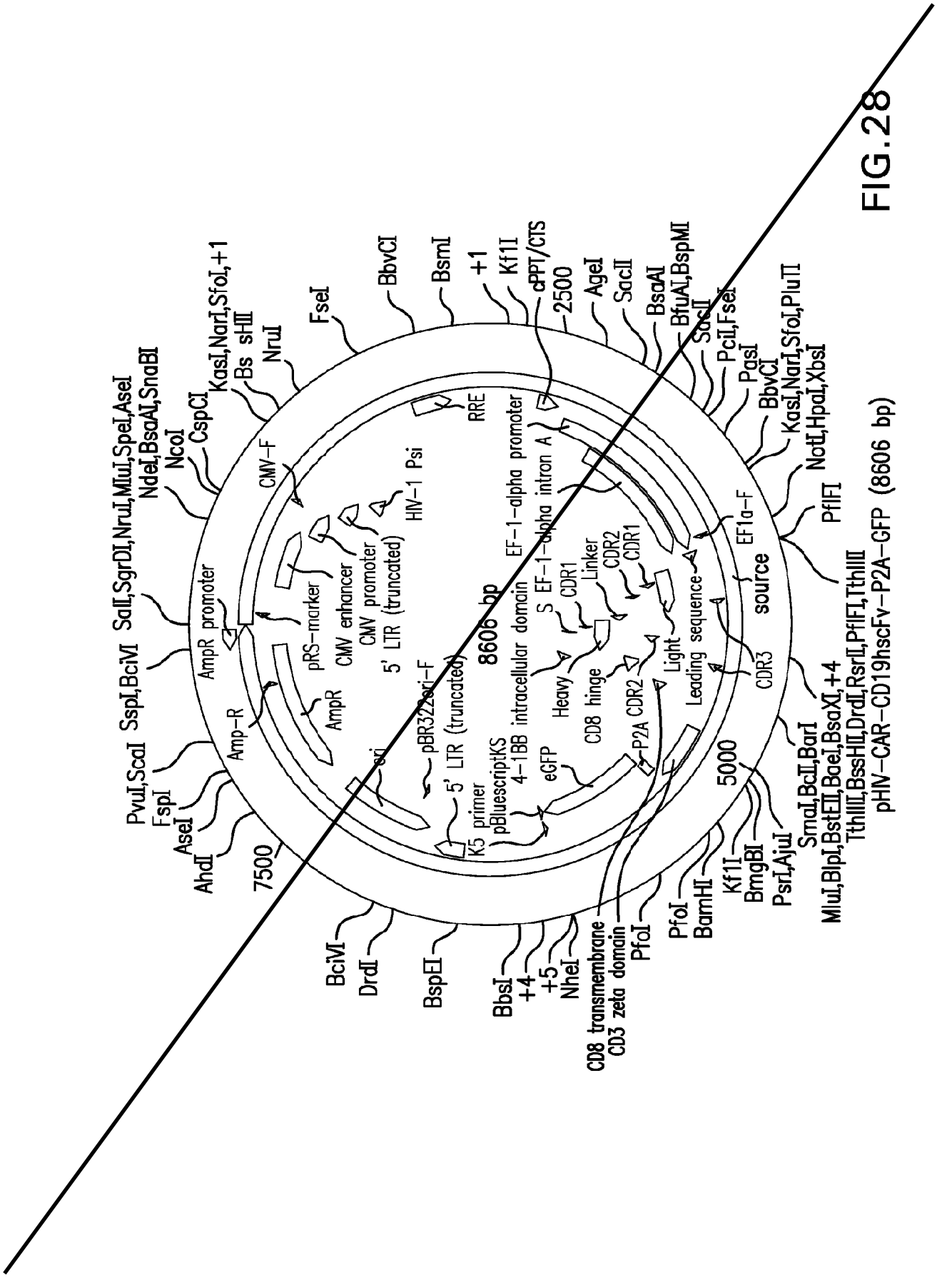

pHV-CAR-CD19hscFv-P2A-GFP (8606 bp)
8606 bp
5000
7500
2500
+1
+4
+5
5' LTR (truncated)
HIV-1 Psi
RRE
cPPT/CTS
EF-1-alpha promoter
EF-1-alpha intron A
CMV-F
CMV promoter
CMV enhancer
pRS-marker
AmpR promoter
AmpR
Amp-R
ori
pBR322ori-F
pBluescriptKS
K5 primer
5' LTR (truncated)
eGFP
4-1BB intracellular domain
CD8 hinge
Heavy
CDR1
Linker
CDR2
CDR1
Light
Leading sequence
CDR3
EF1a-F
source
P2A
CDR2
CD8 transmembrane
CD3 zeta domain
PvuI,ScaI
SspI,BciVI
SalI,SgrDI,NruI,MluI,SpeI,AseI
NdeI,BsaAI,SnaBI
NcoI
CspCI
KasI,NarI,SfoI,+1
Bs sHII
NruI
FseI
BbvCI
BsmI
+1
Kf1I
AgeI
SacII
BsaAI
BfuAI,BspMI
SacII
PciI,FseI
PasI
BbvCI
KasI,NarI,SfoI,PluTI
NotI,HpaI,XbsI
PflFI
SmaI,BclI,BarI
MluI,BlpI,BstEII,BaeI,BsaXI,+4
TthIIII,BssHII,DrdI,RsrII,PflFI,TthIIII
PsrI,AjuI
BmgBI
Kf1I
BamHI
PfoI
PfoI
NheI
+5
+4
BbsI
BspEI
DrdI
BciVI
FspI
AseI
AhdI

FIG.28

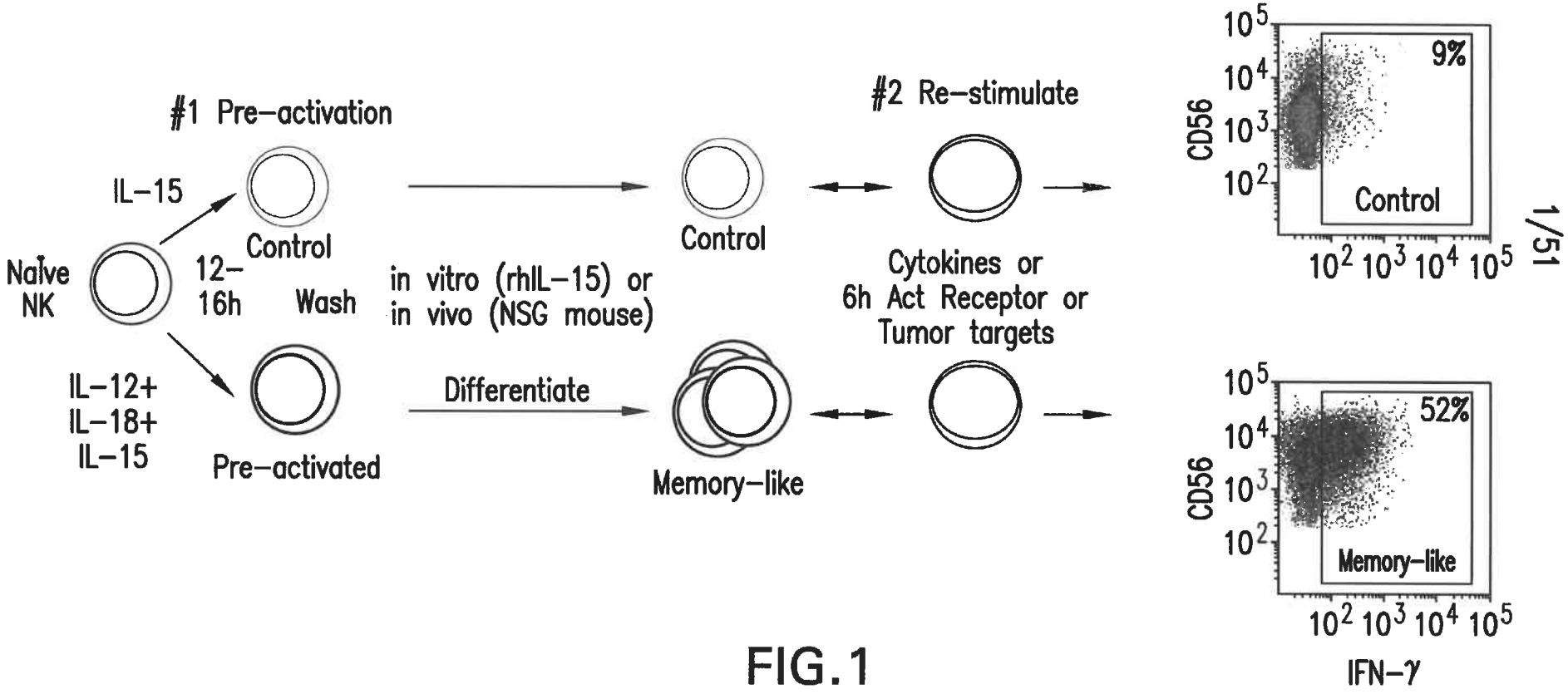

#1 Pre-activation
IL-15
Control
Naïve NK
12-16h
Wash
IL-12+
IL-18+
IL-15
Pre-activated
in vitro (rhIL-15) or
in vivo (NSG mouse)
Differentiate
Control
Memory-like
#2 Re-stimulate
Cytokines or
6h Act Receptor or
Tumor targets
CD56
IFN-γ
9%
Control
52%
Memory-like
1/51

FIG. 1

cNK: Conventional NK cells
CIML NK: Cytokine-induced memory-like NK cells

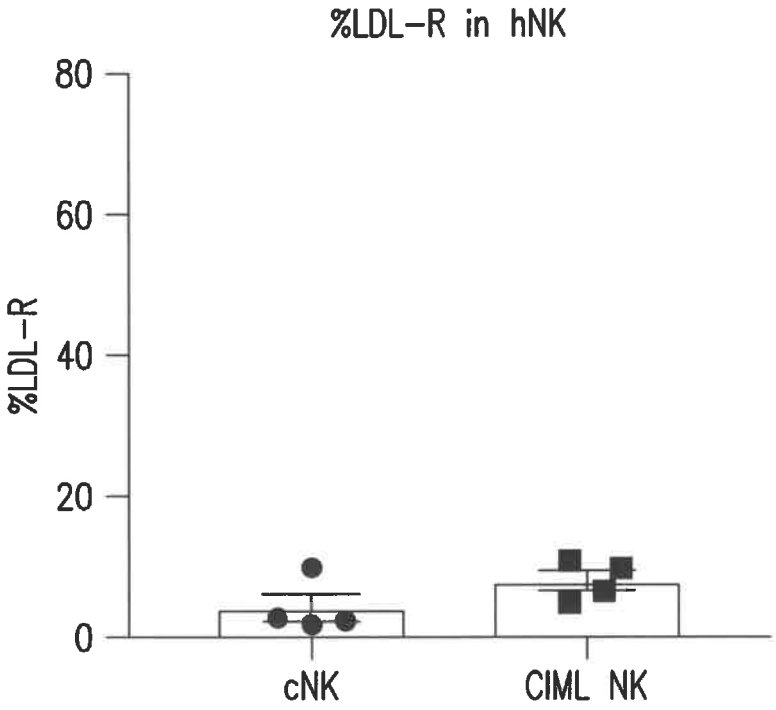


FIG.2A

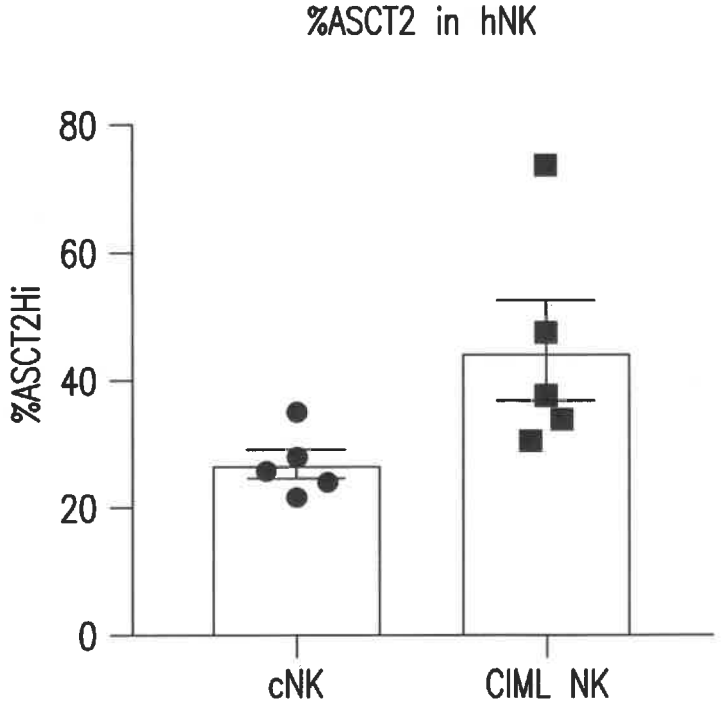


FIG.2B

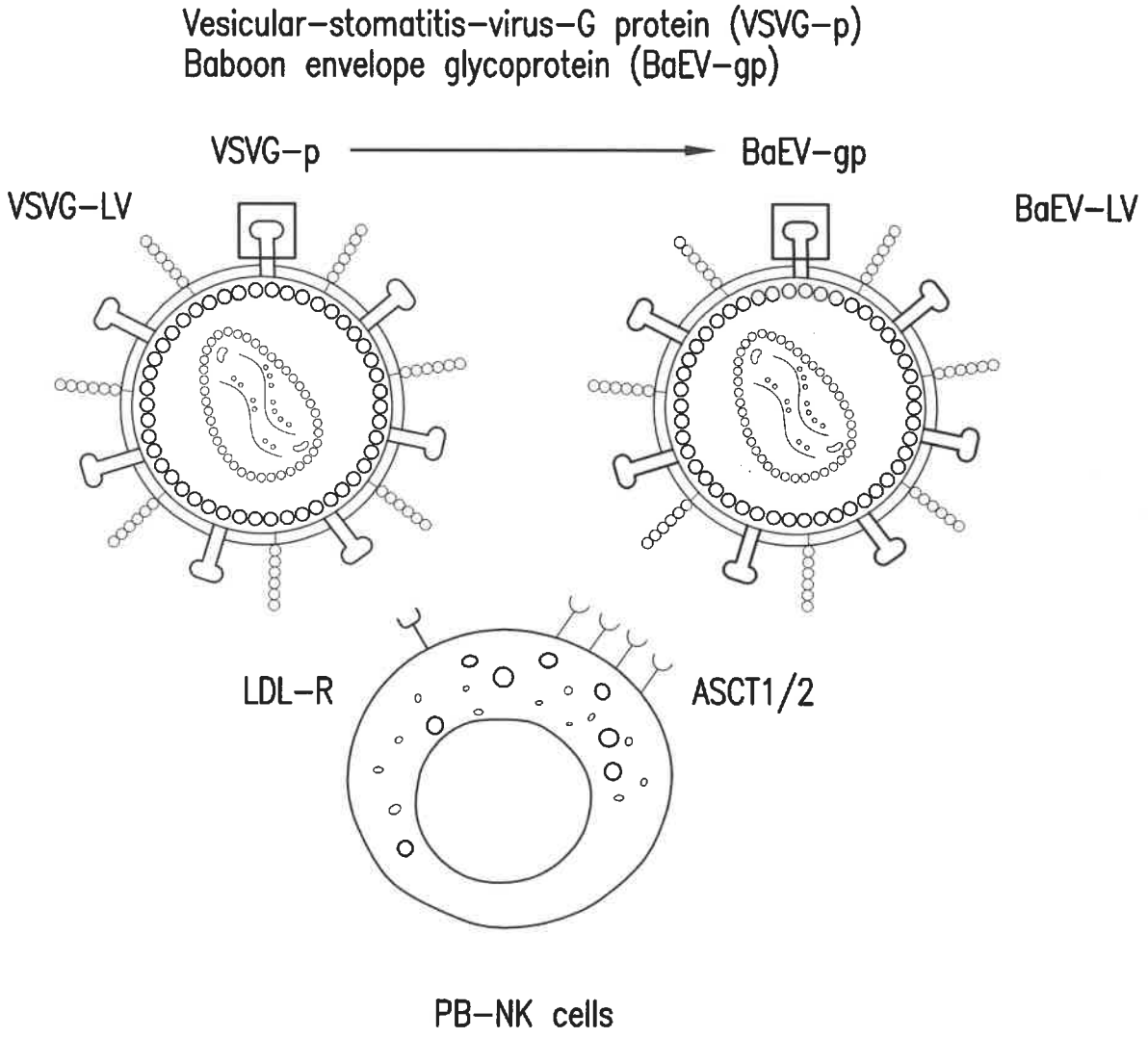

Vesicular-stomatitis-virus-G protein (VSVG-p)
Baboon envelope glycoprotein (BaEV-gp)
VSVG-p
BaEV-gp
VSVG-LV
BaEV-LV
LDL-R
ASCT1/2
PB-NK cells

FIG.2C

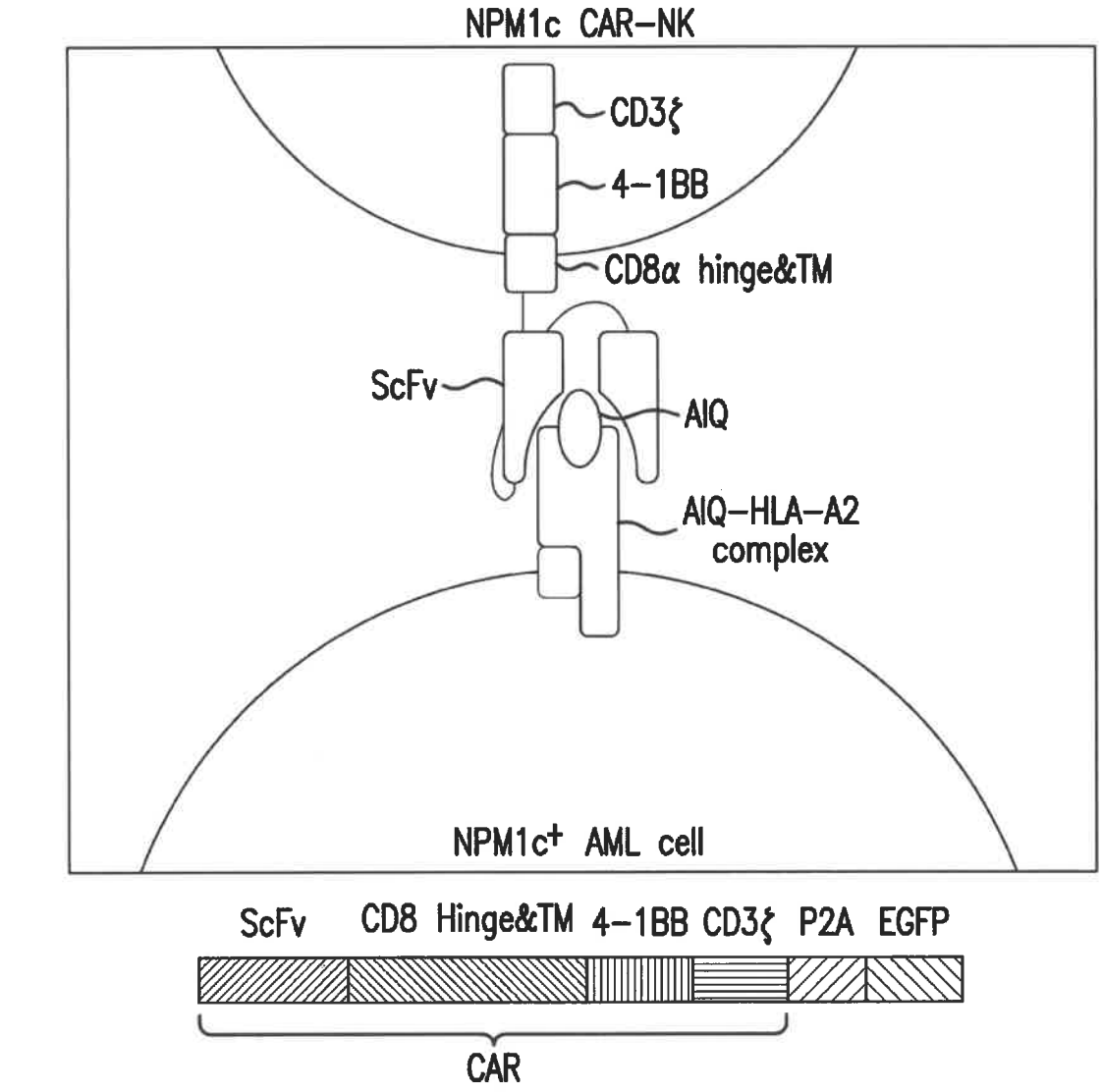

NPM1c CAR-NK
CD3ζ
4-1BB
CD8α hinge&TM
ScFv
AIQ
AIQ-HLA-A2 complex
NPM1c+ AML cell
ScFv
CD8 Hinge&TM
4-1BB
CD3ζ
P2A
EGFP
CAR

FIG.3A

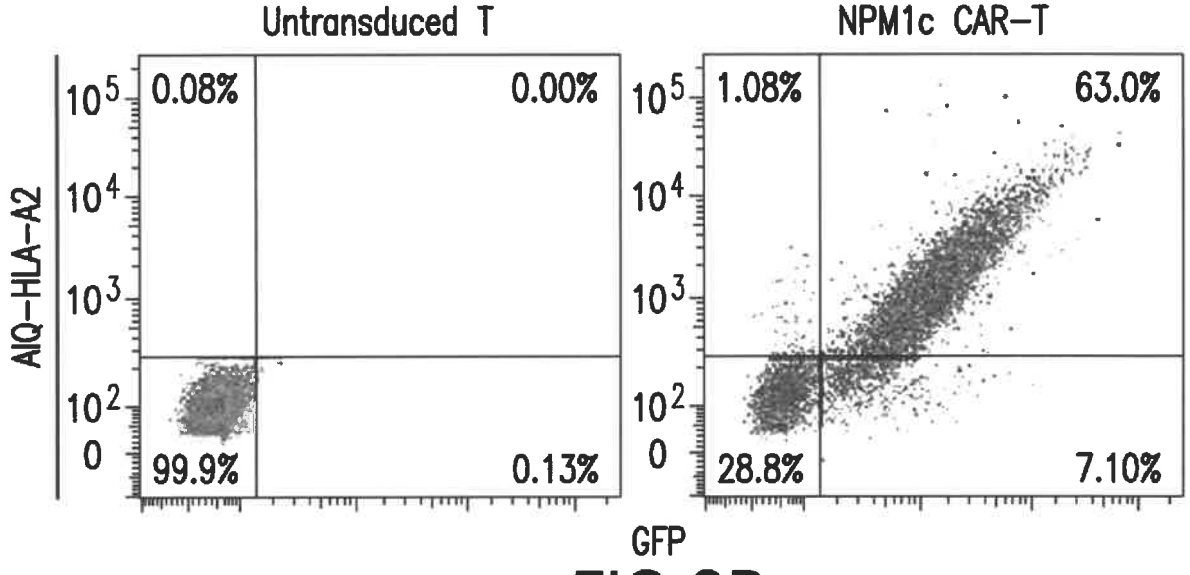
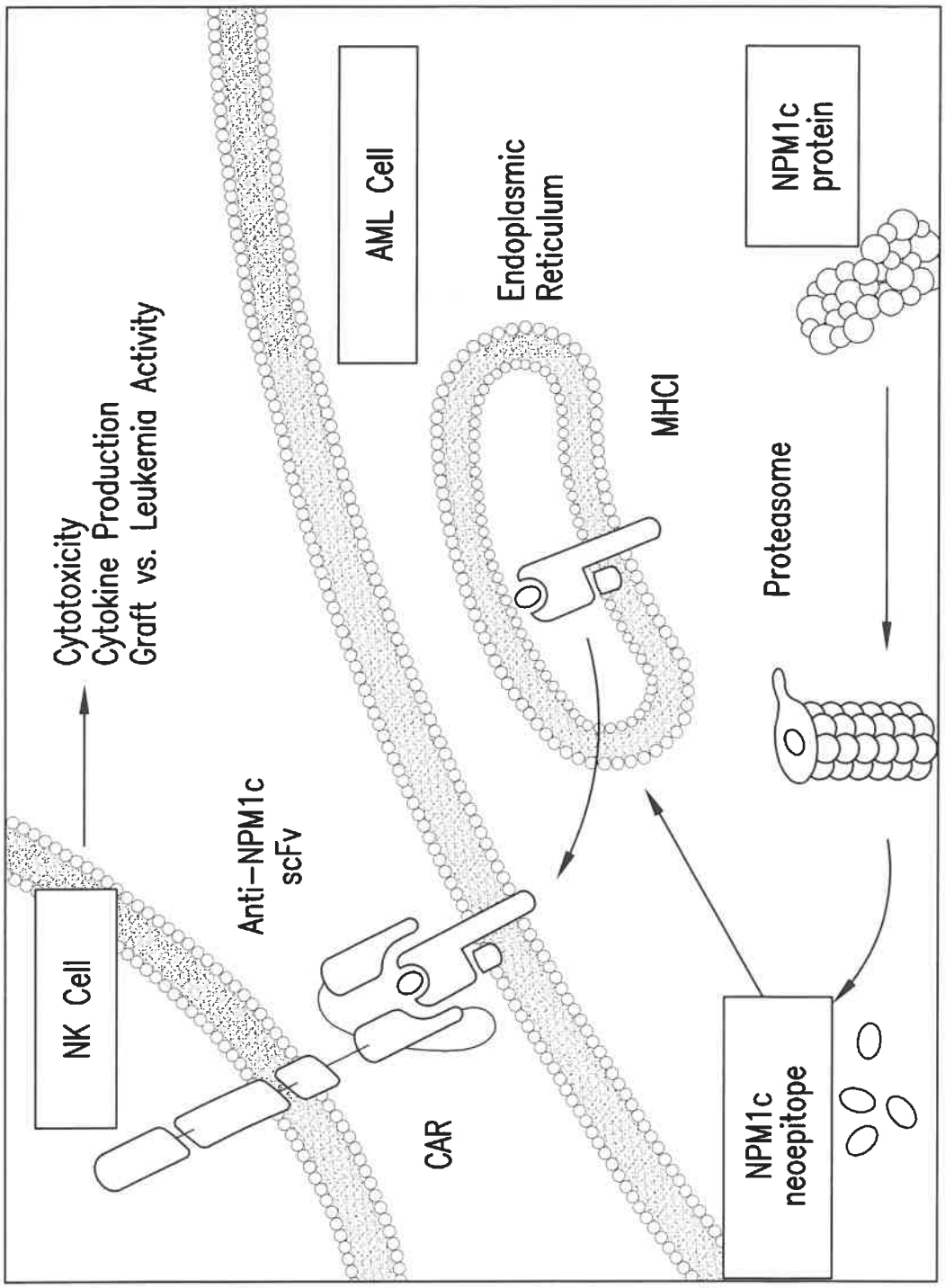

Untransduced T
NPM1c CAR-T
AIQ-HLA-A2
GFP
0.08%
0.00%
99.9%
0.13%
1.08%
63.0%
28.8%
7.10%

FIG.3B



CAR targeting a neoepitope derived from NPM1c protein

FIG.4

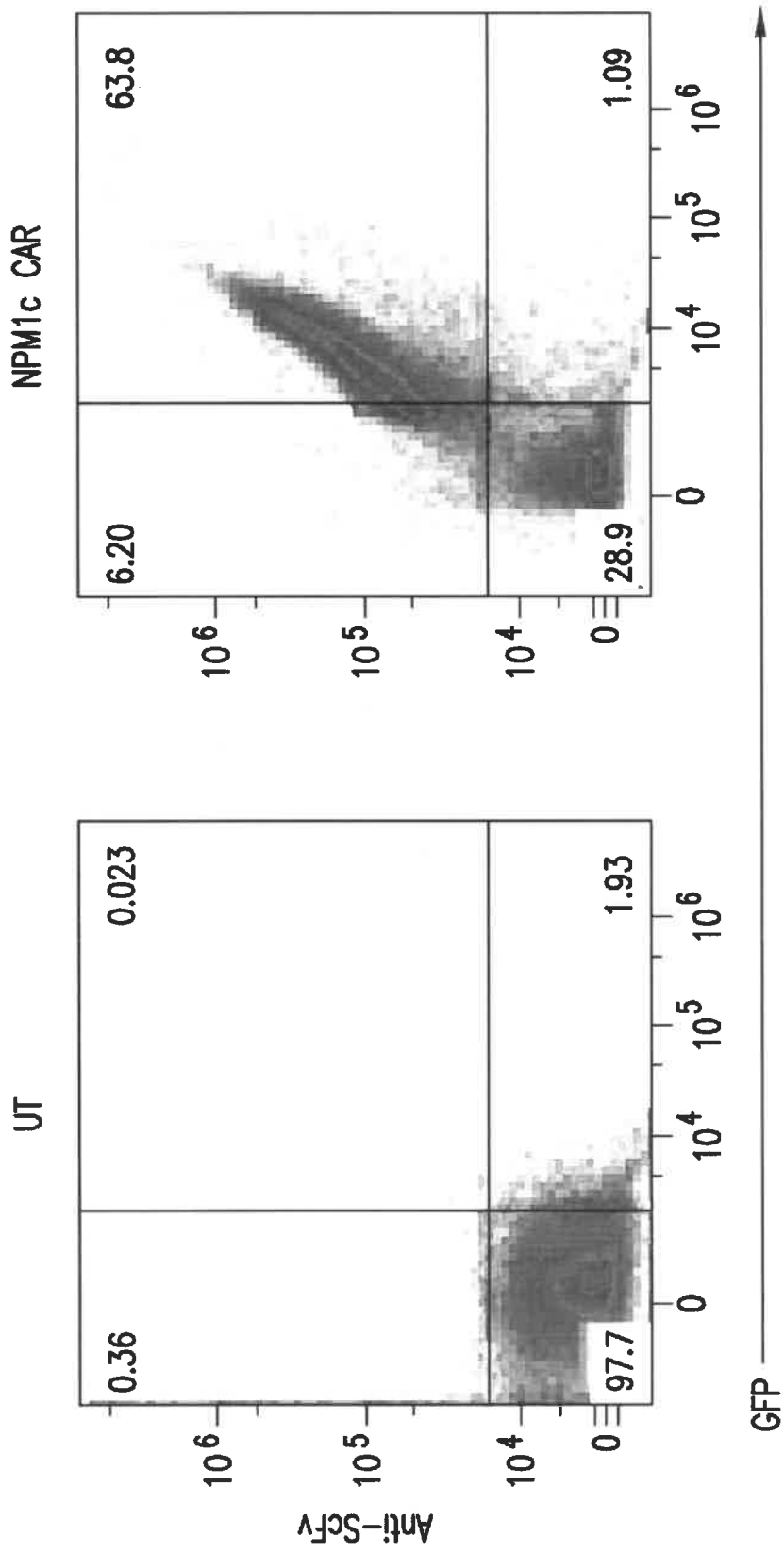

UT
NPM1c CAR
0.36
0.023
97.7
1.93
6.20
63.8
28.9
1.09
Anti-ScFv
GFP
0
10^4
10^5
10^6

FIG.5

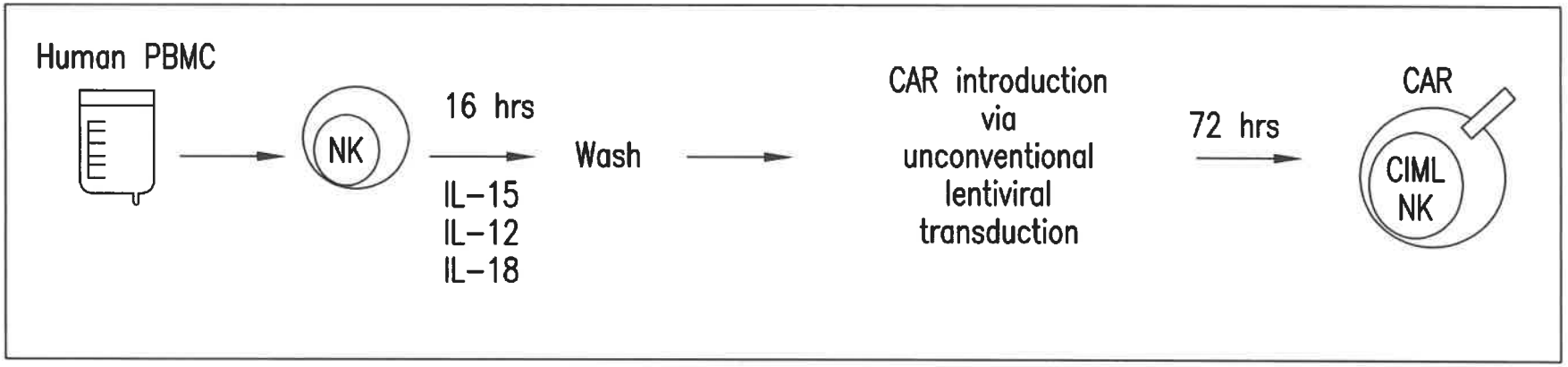

Human PBMC
NK
16 hrs
IL-15
IL-12
IL-18
Wash
CAR introduction
via
unconventional
lentiviral
transduction
72 hrs
CAR
CIML
NK

FIG.6A

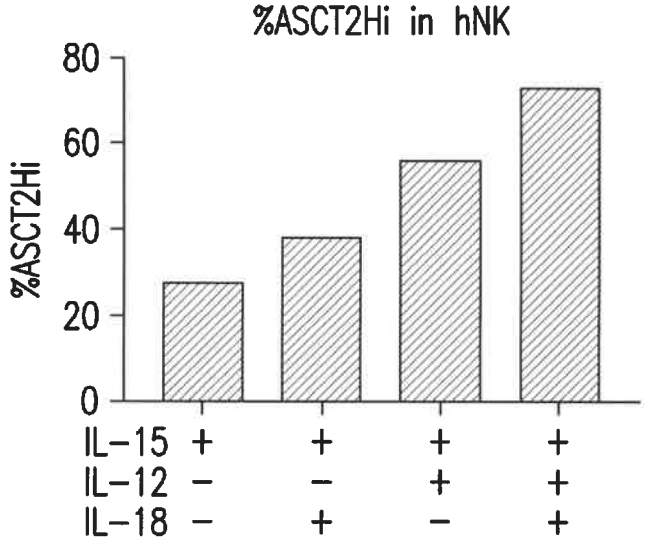

%ASCT2Hi in hNK
%ASCT2Hi
80
60
40
20
0
IL-15 + + + +
IL-12 - - + +
IL-18 - + - +

FIG.6B

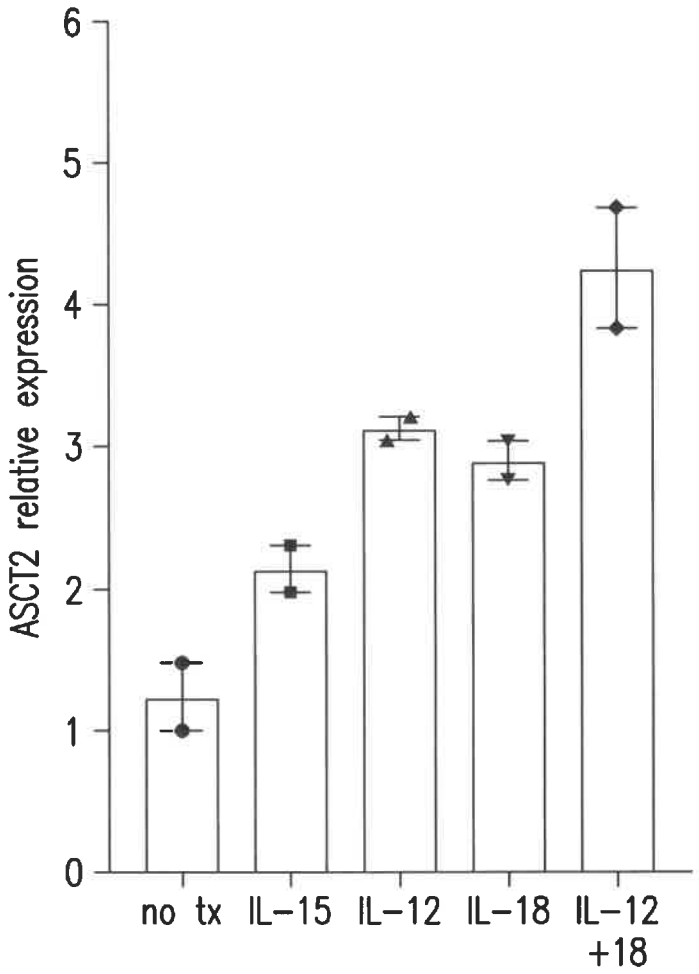


FIG.6C

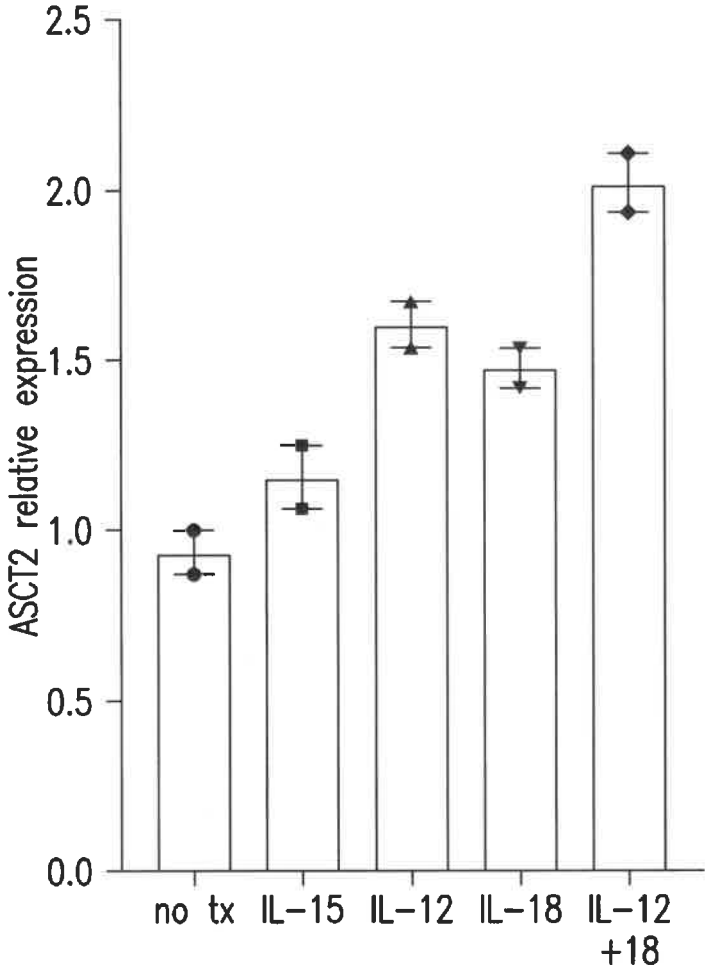


FIG.6D

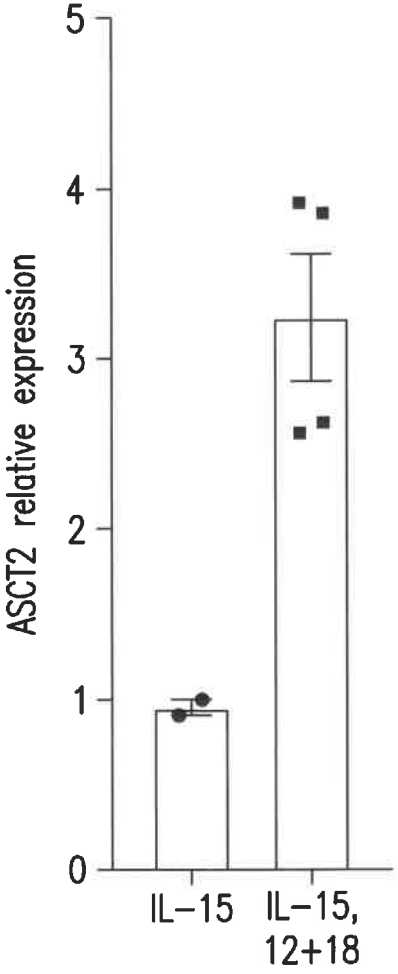


FIG.6E

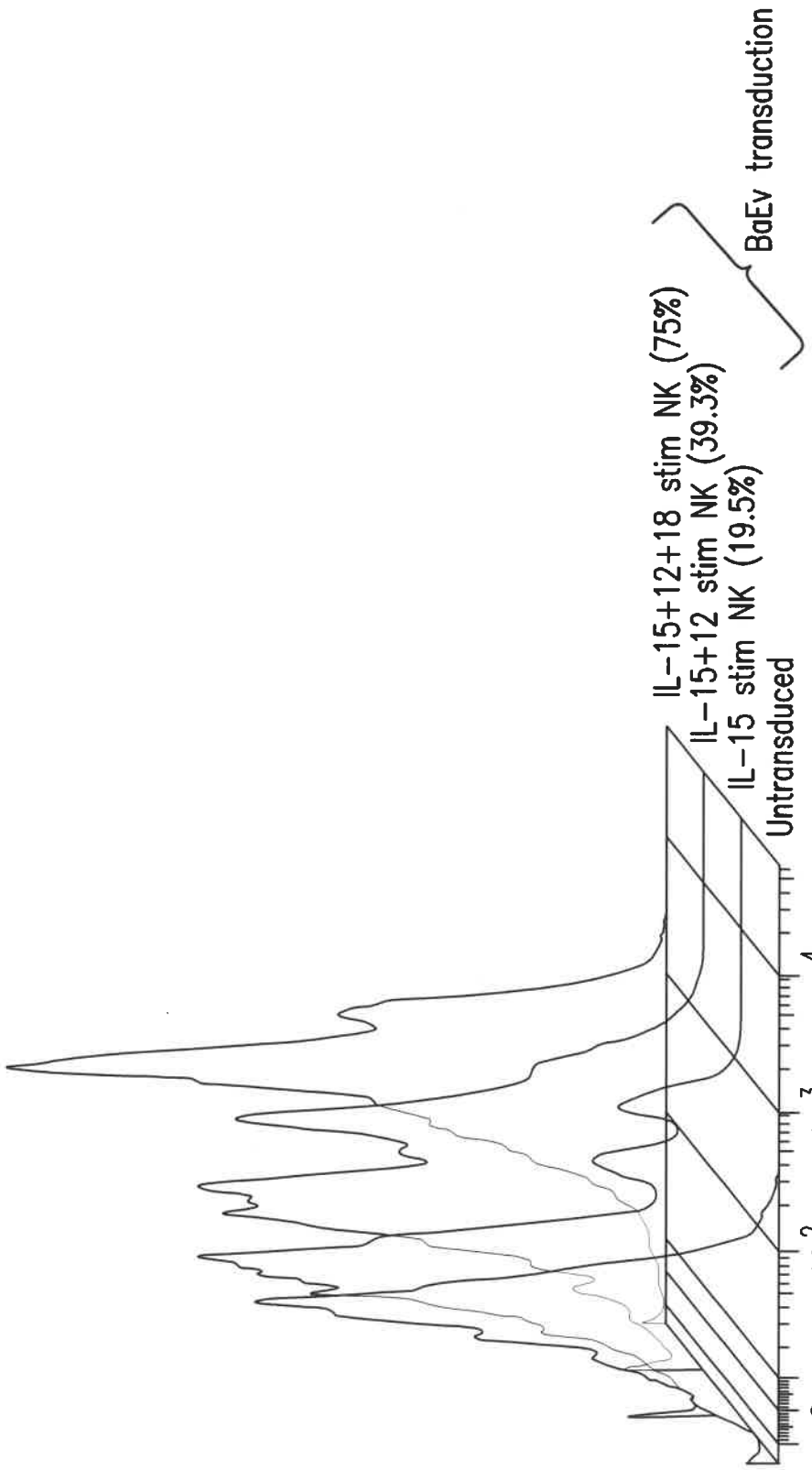

IL-15+12+18 stim NK (75%)
IL-15+12 stim NK (39.3%)
IL-15 stim NK (19.5%)
Untransduced
BaEv transduction
0
10^2
10^3
10^4
GFP (transduction reporter)

FIG.6F

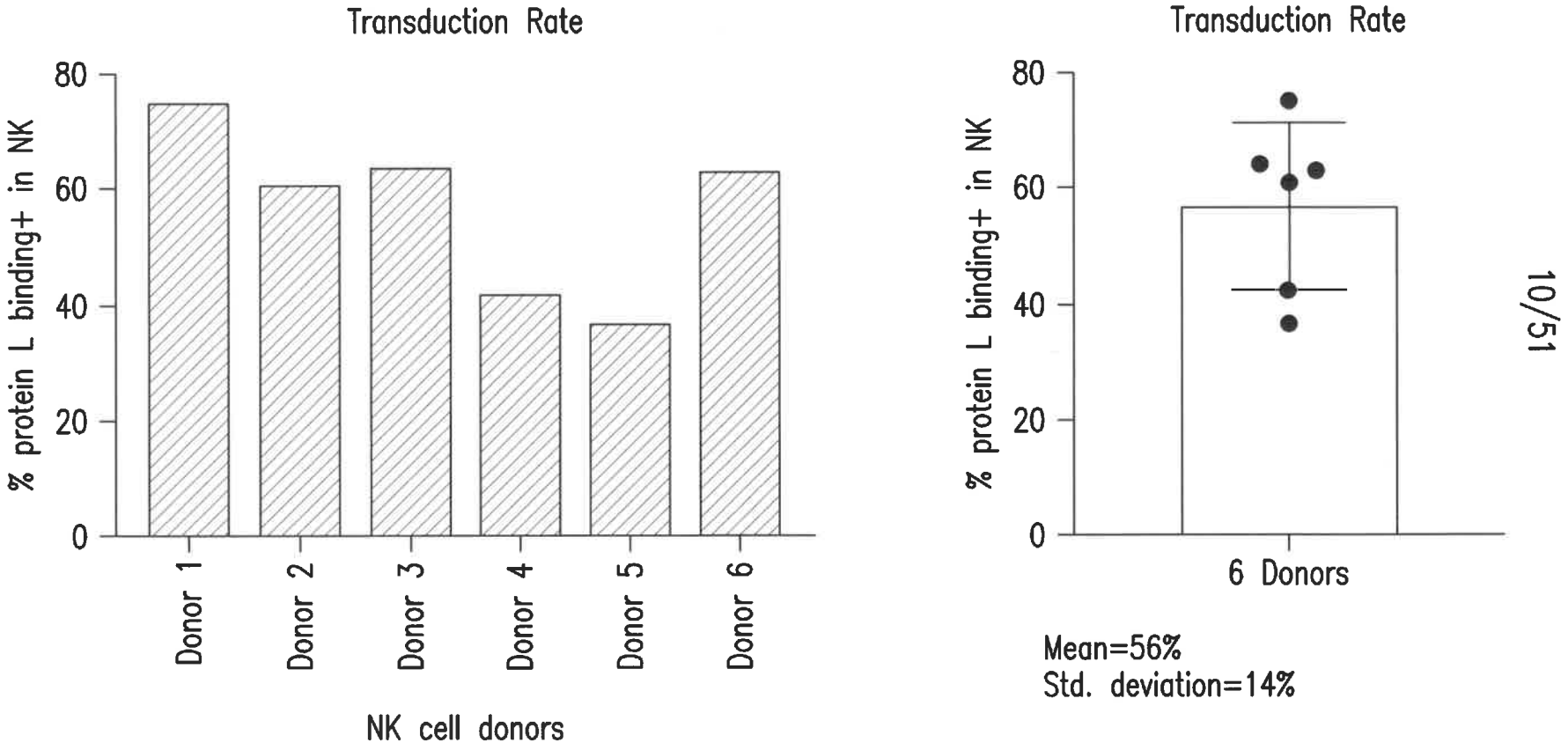

Transduction Rate
% protein L binding+ in NK
80
60
40
20
0
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
NK cell donors
Transduction Rate
% protein L binding+ in NK
80
60
40
20
0
6 Donors
Mean=56%
Std. deviation=14%

FIG.7A

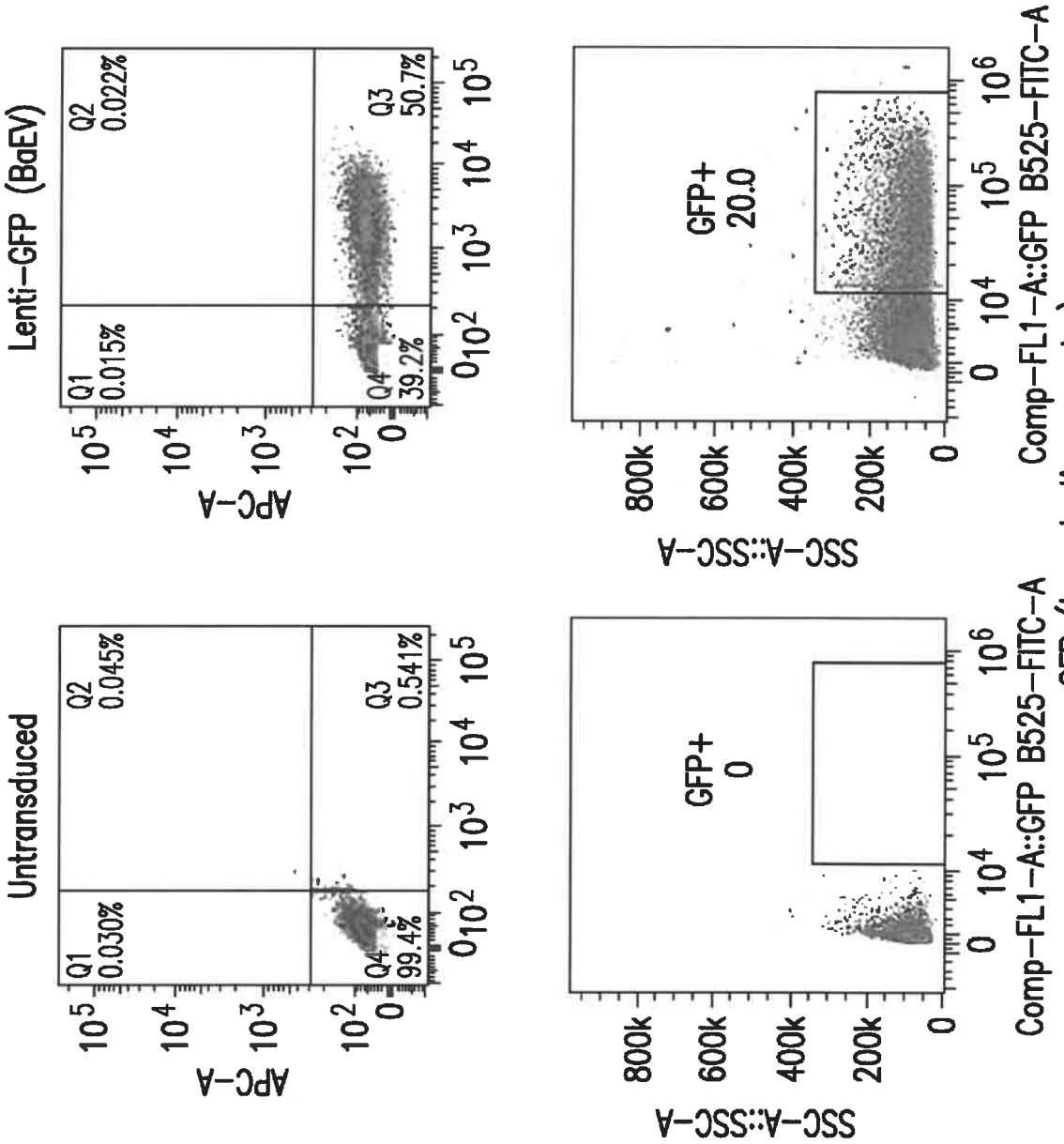

Lenti-GFP (BaEV)
Untransduced
Human NK from PBMC
Mouse NK from spleen
APC-A
Q1 0.030%
Q2 0.045%
Q4 99.4%
Q3 0.541%
Q1 0.015%
Q2 0.022%
Q4 39.2%
Q3 50.7%
SSC-A::SSC-A
GFP+ 0
GFP+ 20.0
Comp-FL1-A::GFP B525-FITC-A
GFP (transduction reporter)

FIG.7B

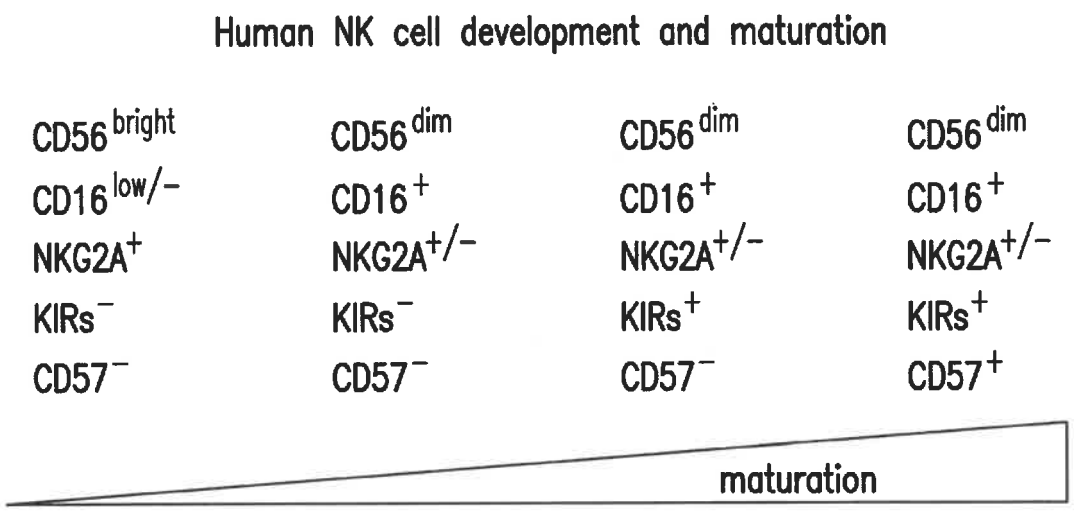


FIG.7C

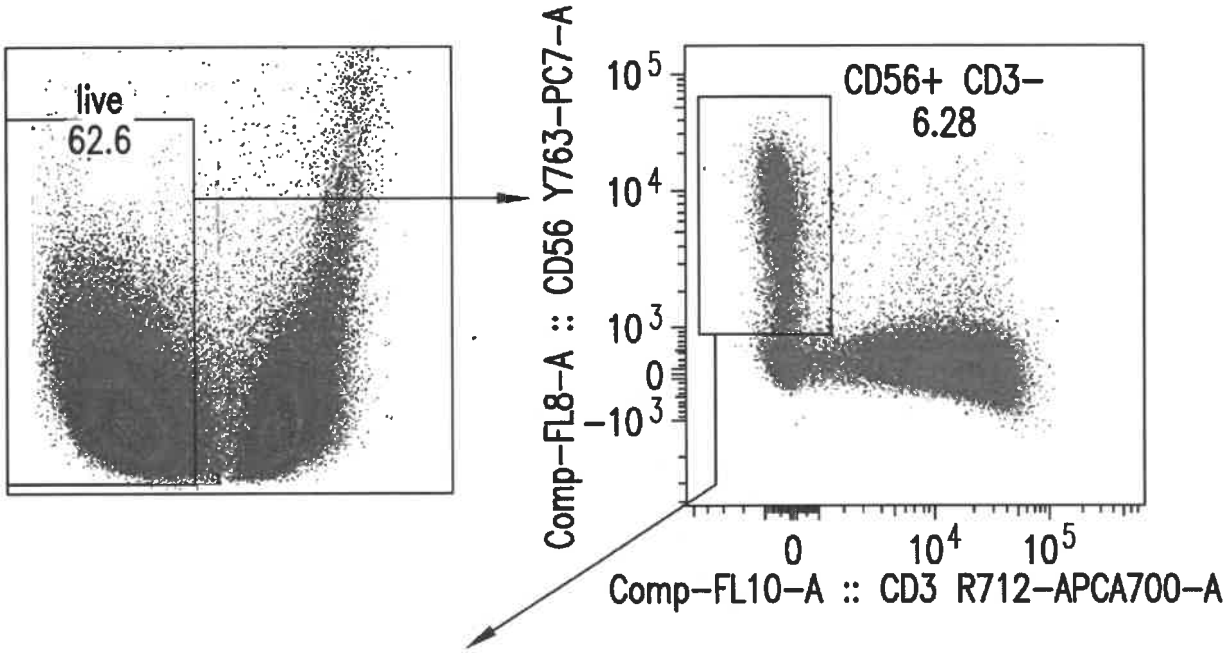


FIG.7D-2

FIG.7D-1

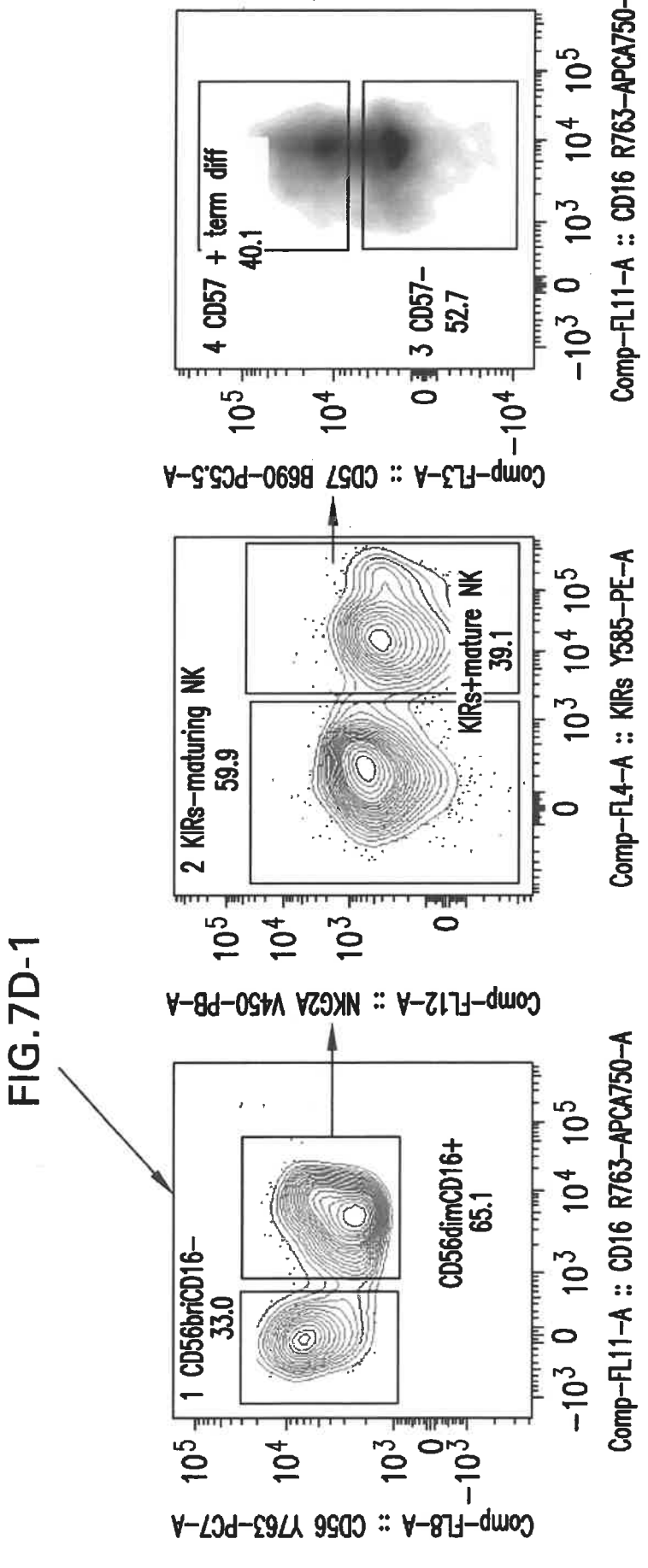


FIG.7D-1

FIG.7D-2

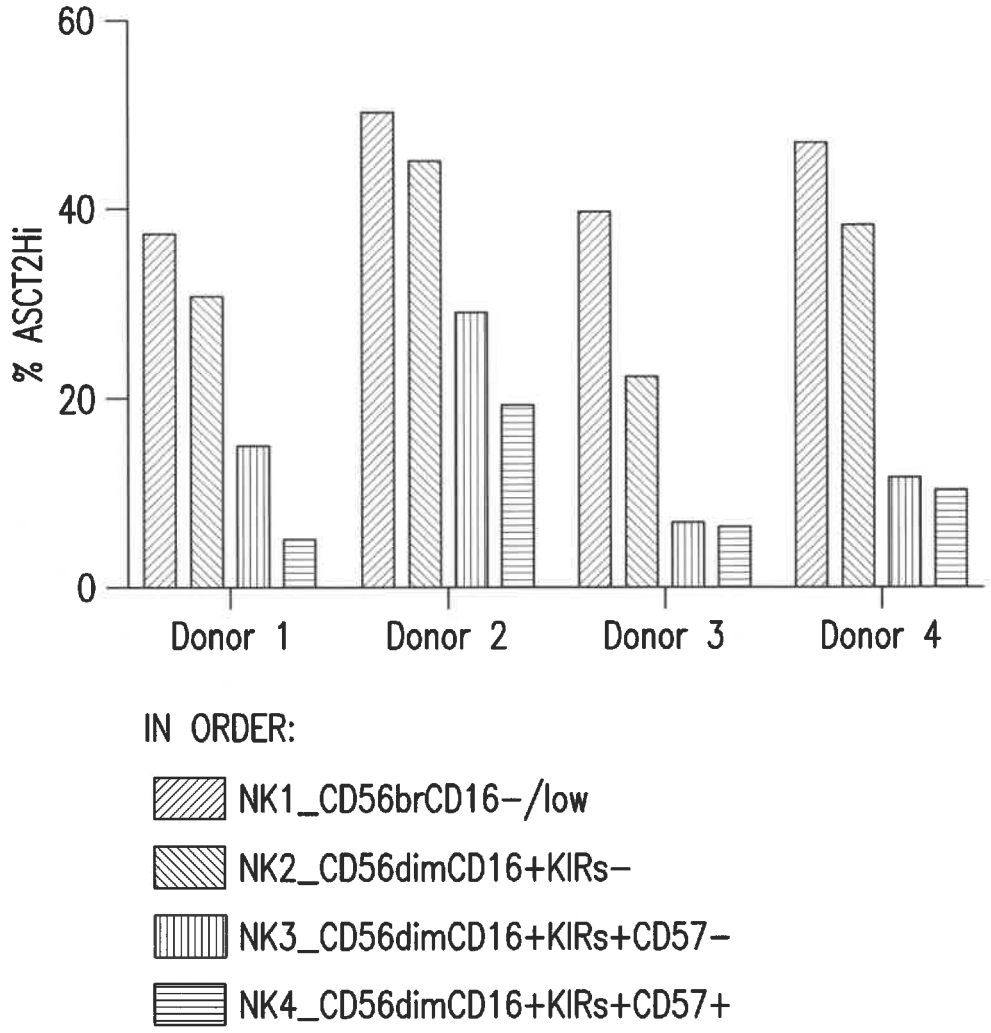

60
40
20
0
% ASCT2Hi
Donor 1
Donor 2
Donor 3
Donor 4
IN ORDER:
NK1_CD56brCD16-/low
NK2_CD56dimCD16+KIRs-
NK3_CD56dimCD16+KIRs+CD57-
NK4_CD56dimCD16+KIRs+CD57+

FIG.7E

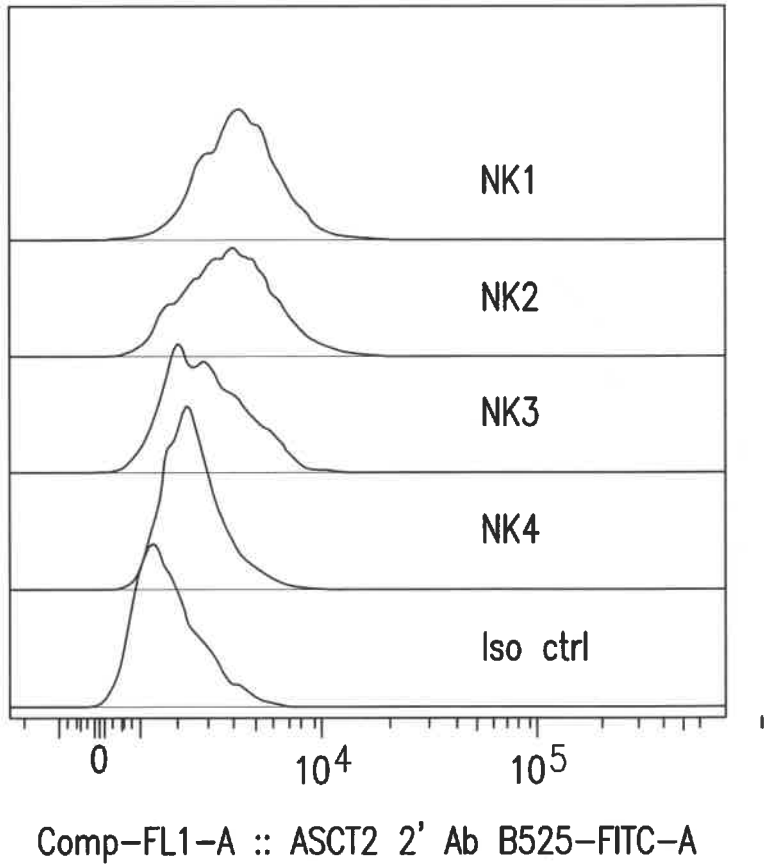

NK1
NK2
NK3
NK4
Iso ctrl
0
10^4
10^5
Comp-FL1-A :: ASCT2 2' Ab B525-FITC-A

FIG.7F

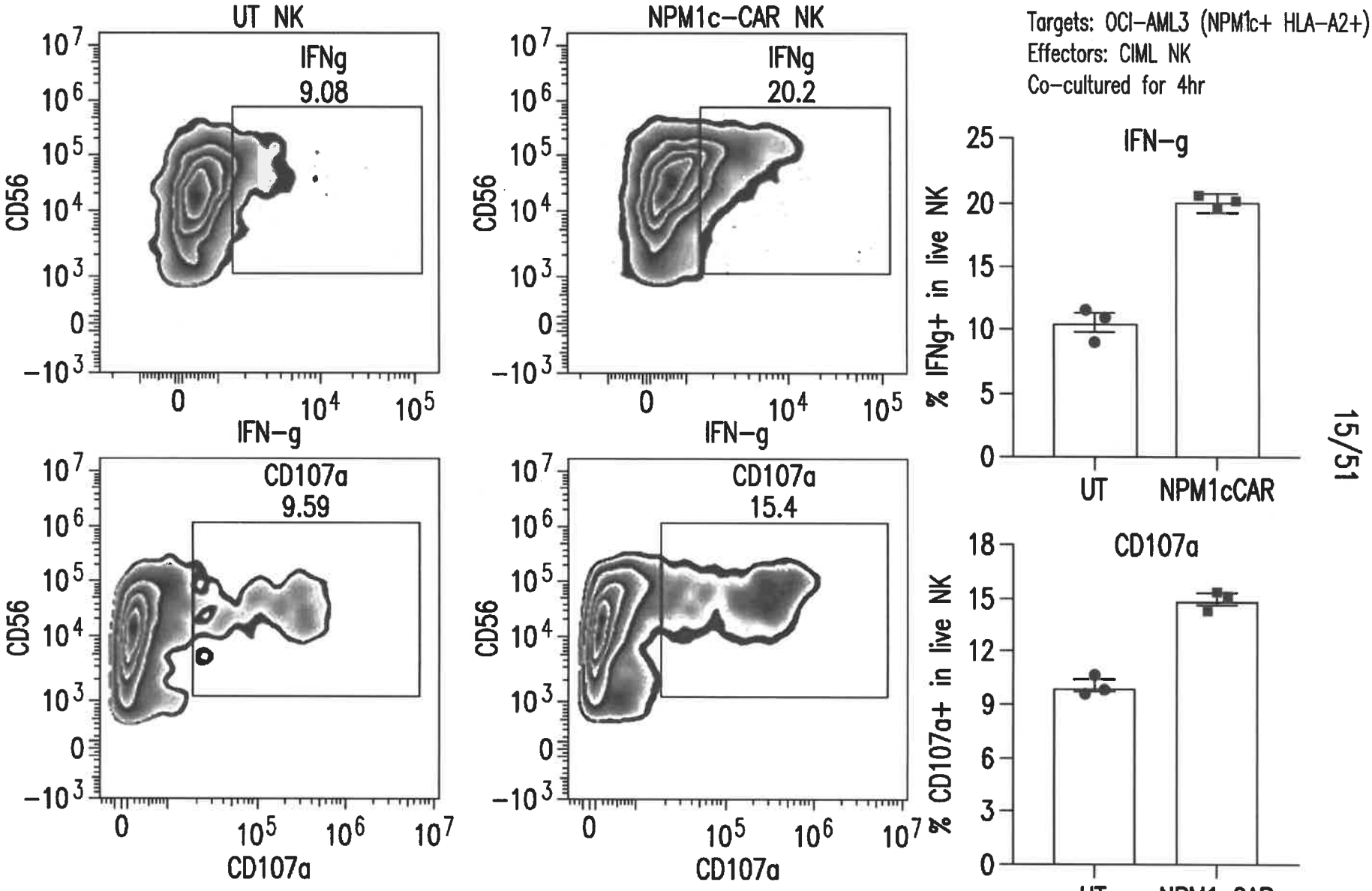

UT NK
IFNg
9.08
CD56
IFN-g
CD107a
9.59
CD107a
NPM1c-CAR NK
IFNg
20.2
CD56
IFN-g
CD107a
15.4
CD107a
Targets: OCI-AML3 (NPM1c+ HLA-A2+)
Effectors: CIML NK
Co-cultured for 4hr
IFN-g
% IFNg+ in live NK
UT
NPM1cCAR
CD107a
% CD107a+ in live NK
UT
NPM1cCAR

FIG.8A

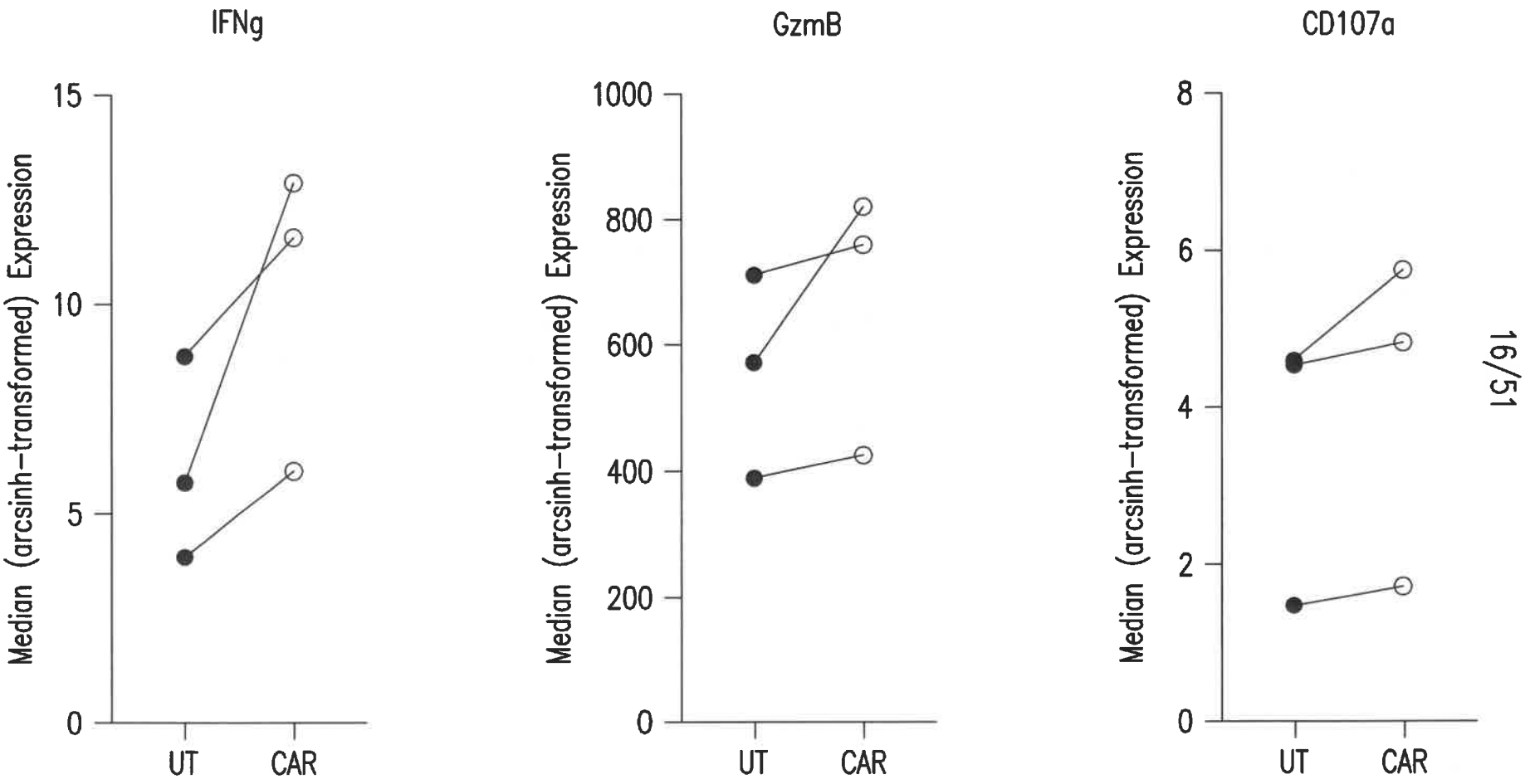

IFNg
GzmB
CD107a
Median (arcsinh−transformed) Expression
15
10
5
0
1000
800
600
400
200
8
6
4
2
UT
CAR

FIG.8B

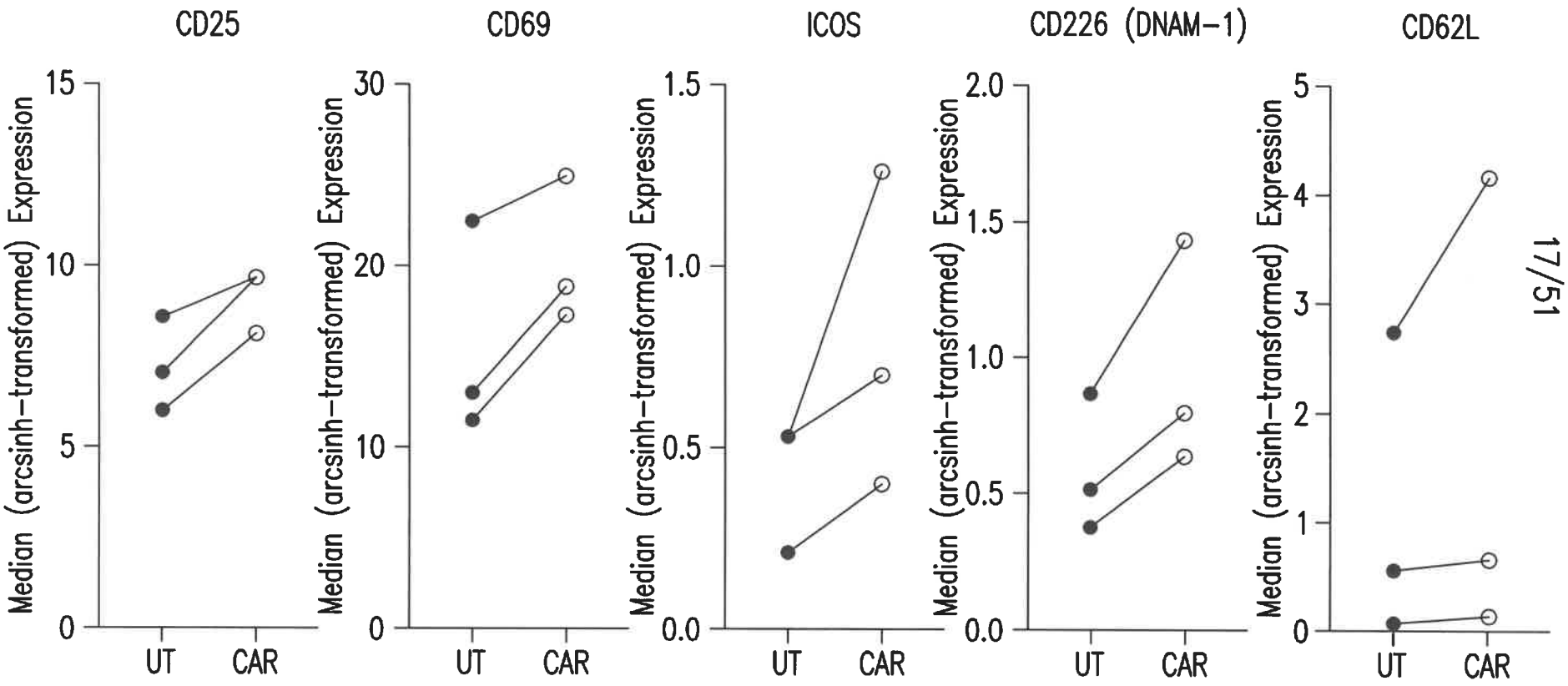

CD25
CD69
ICOS
CD226 (DNAM-1)
CD62L
Median (arcsinh-transformed) Expression
15
10
5
0
30
20
10
0
1.5
1.0
0.5
0.0
2.0
1.5
1.0
0.5
0.0
5
4
3
2
1
0
UT
CAR

FIG.8C

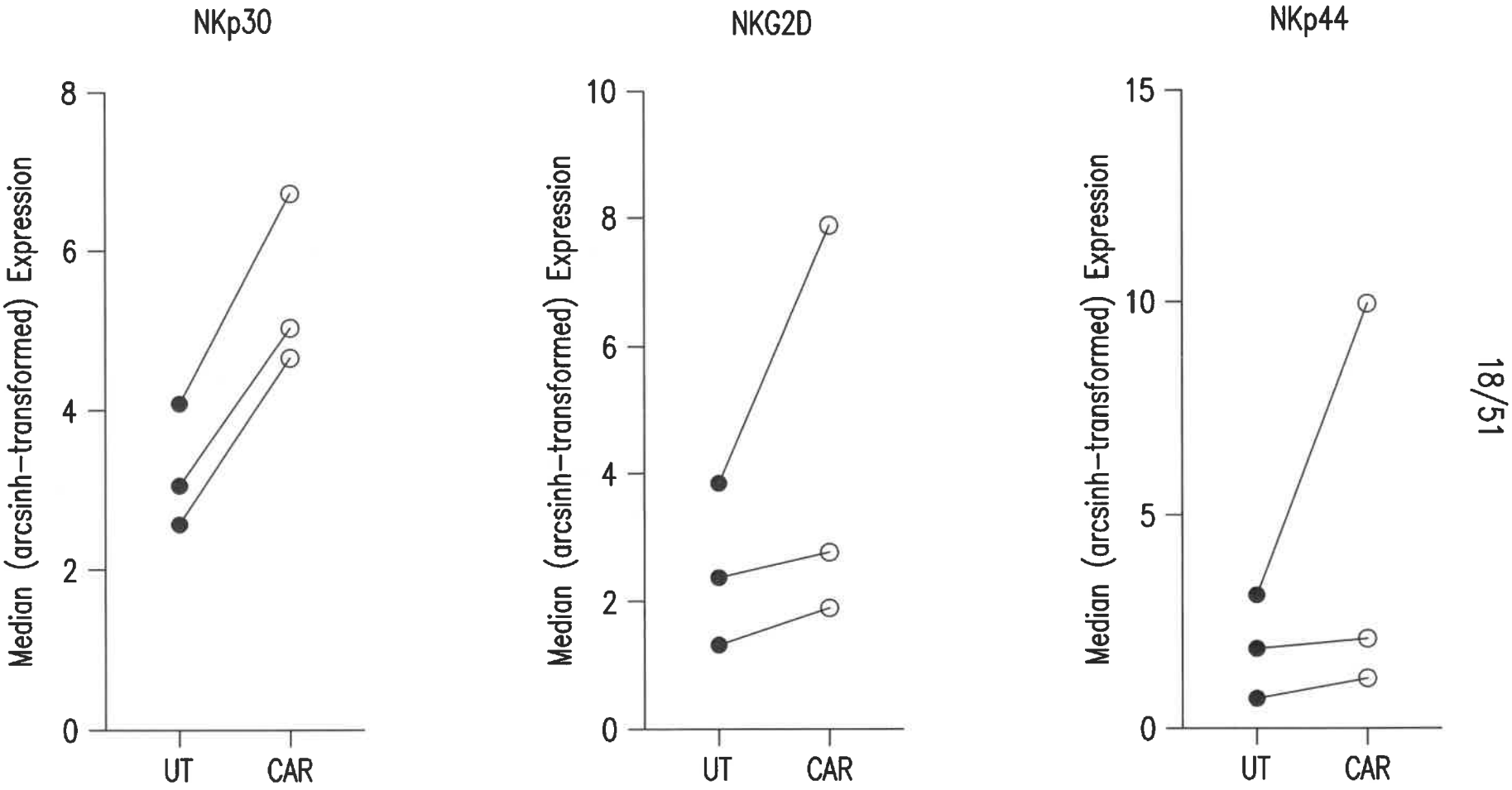

NKp30
NKG2D
NKp44
Median (arcsinh-transformed) Expression
0
2
4
6
8
10
5
15
UT
CAR

FIG.8D

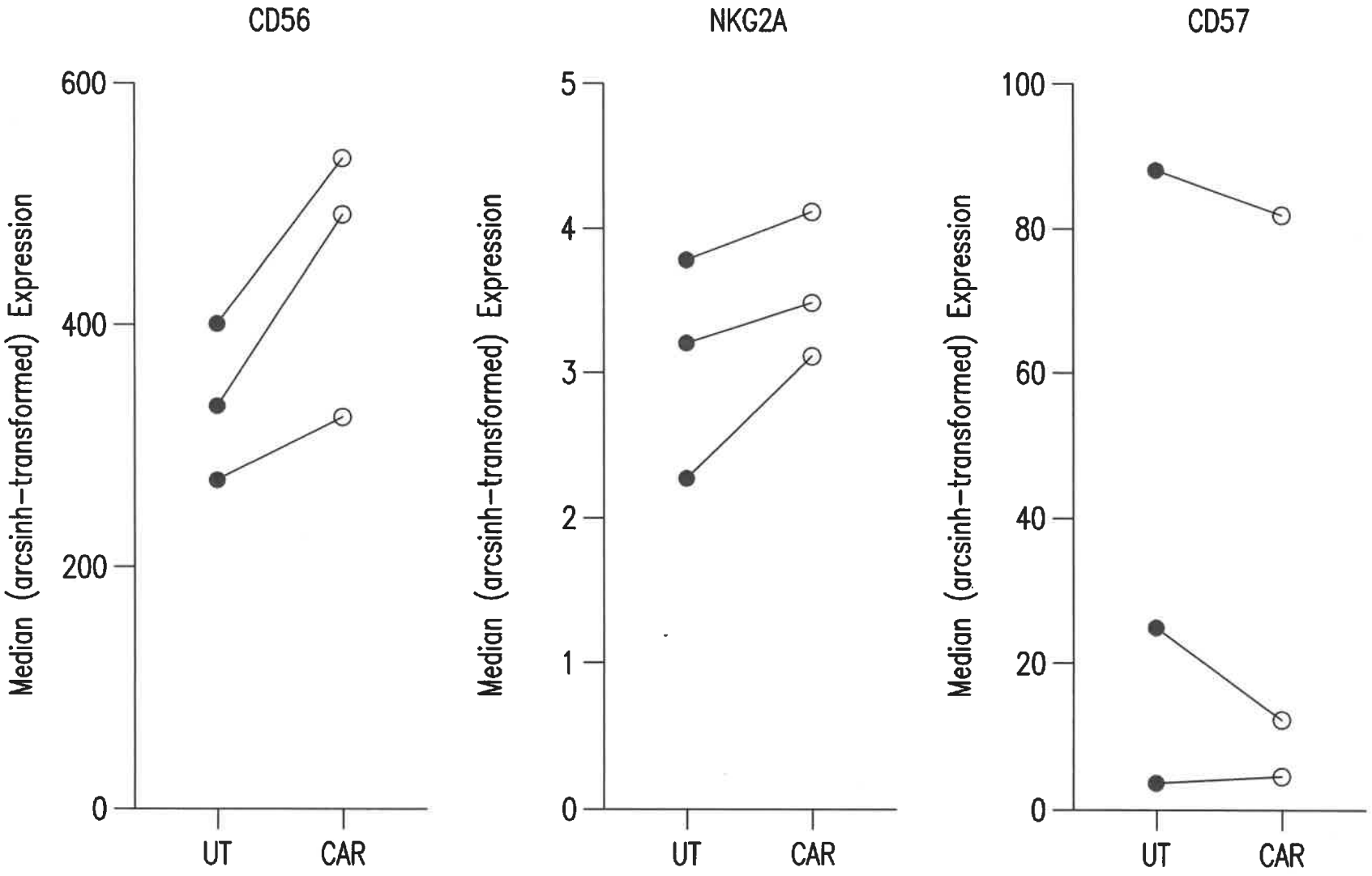

CD56
NKG2A
CD57
Median (arcsinh-transformed) Expression
600
400
200
0
5
4
3
2
1
100
80
60
40
20
UT
CAR

FIG.8E

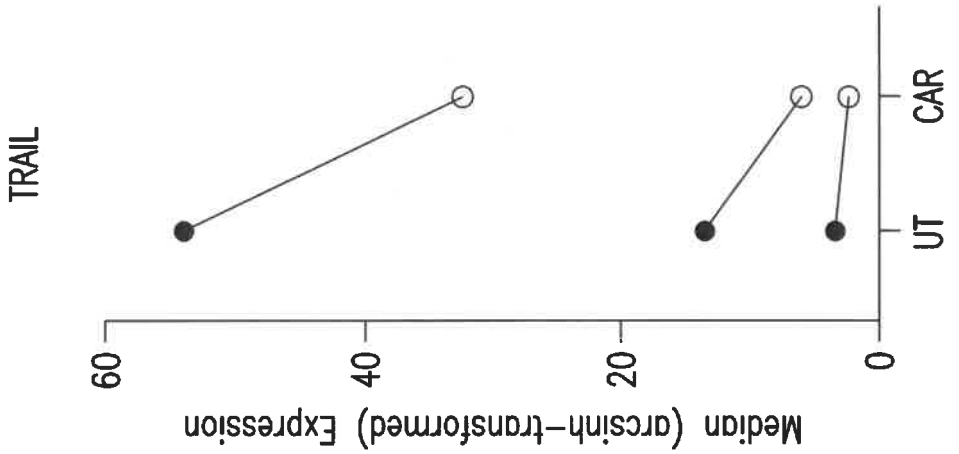

TRAIL
Median (arcsinh−transformed) Expression
0
20
40
60
UT
CAR

FIG.8G

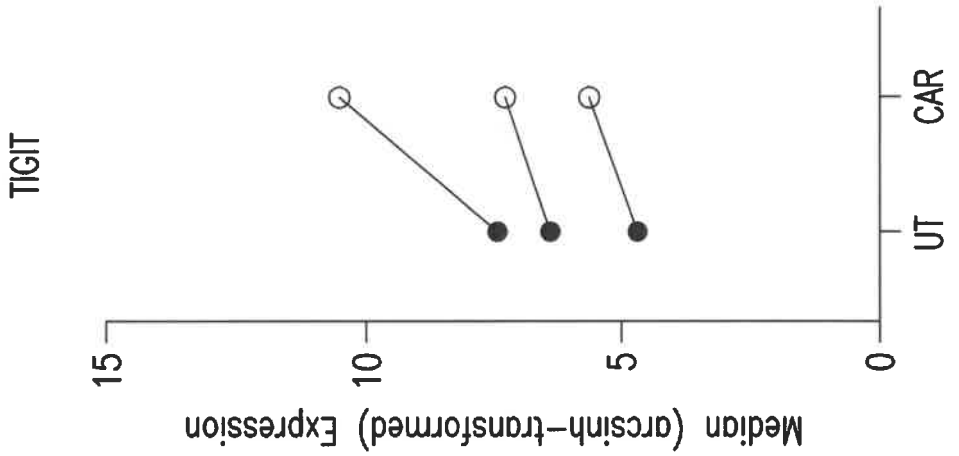

TIGIT
Median (arcsinh−transformed) Expression
0
5
10
15
UT
CAR

FIG.8F

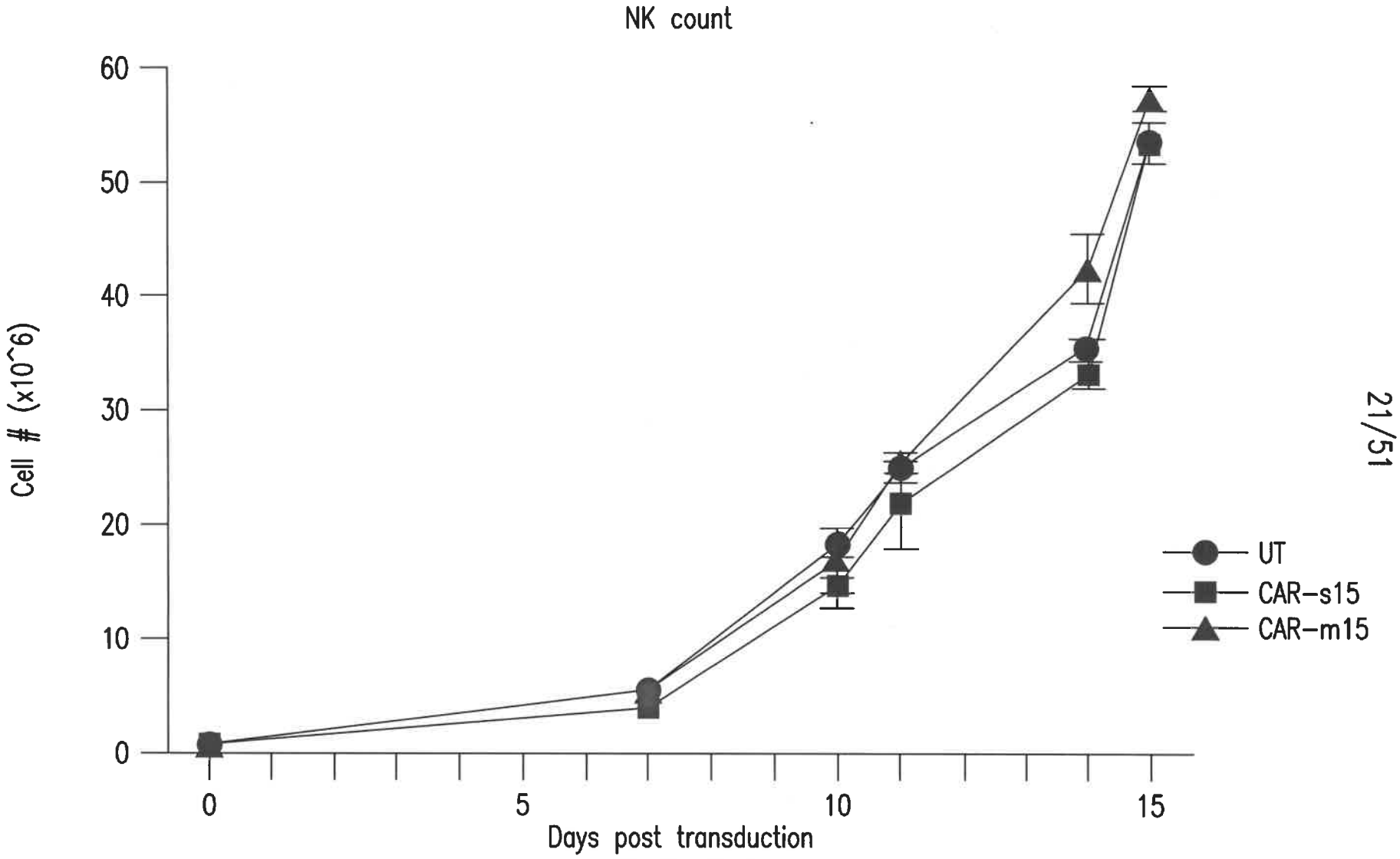

NK count
60
50
40
30
20
10
0
Cell # (x10^6)
0
5
10
15
Days post transduction
UT
CAR-s15
CAR-m15

FIG.9A

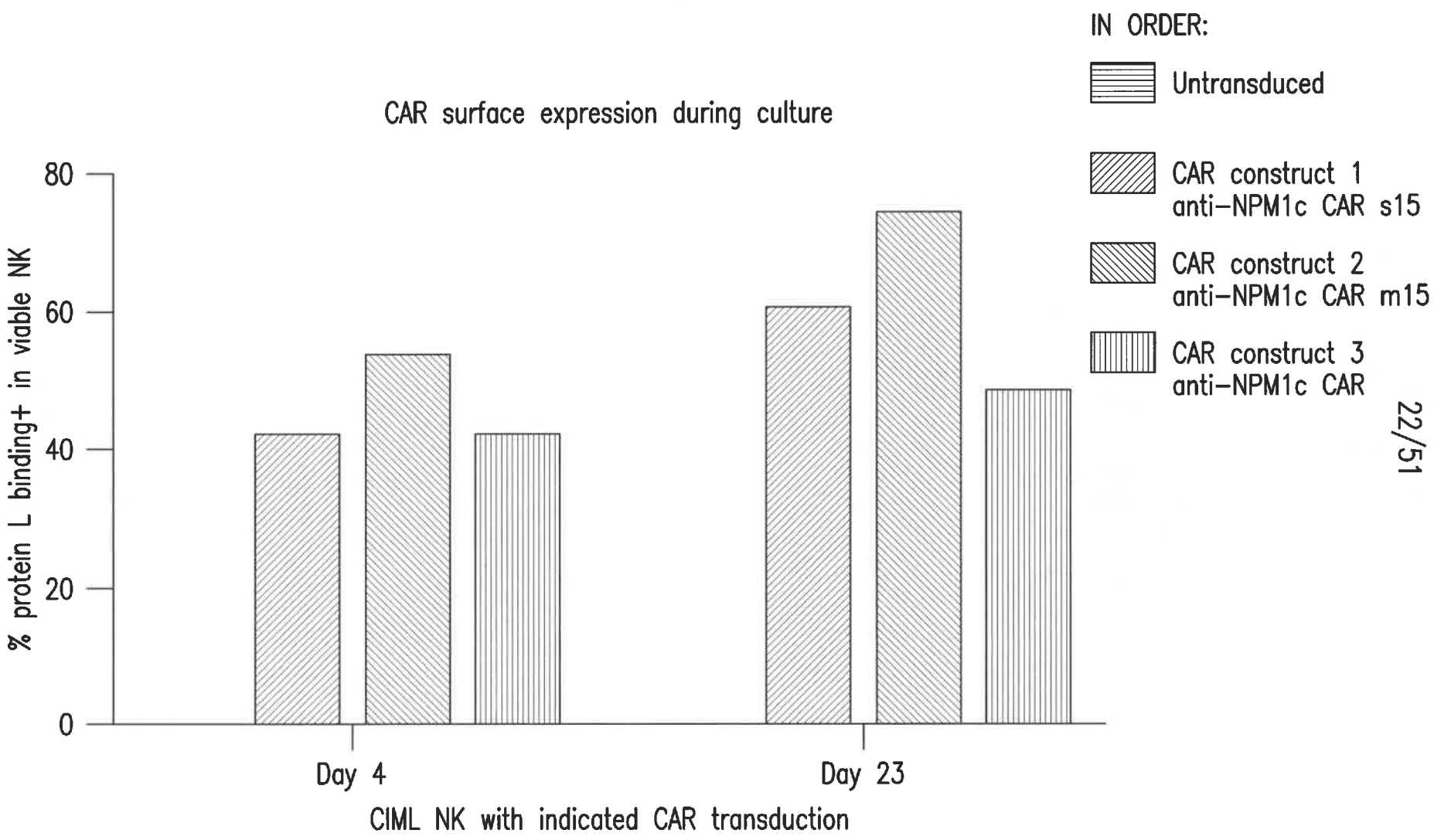

CAR surface expression during culture
% protein L binding+ in viable NK
80
60
40
20
0
Day 4
Day 23
CIML NK with indicated CAR transduction
IN ORDER:
Untransduced
CAR construct 1
anti-NPM1c CAR s15
CAR construct 2
anti-NPM1c CAR m15
CAR construct 3
anti-NPM1c CAR

FIG.9B

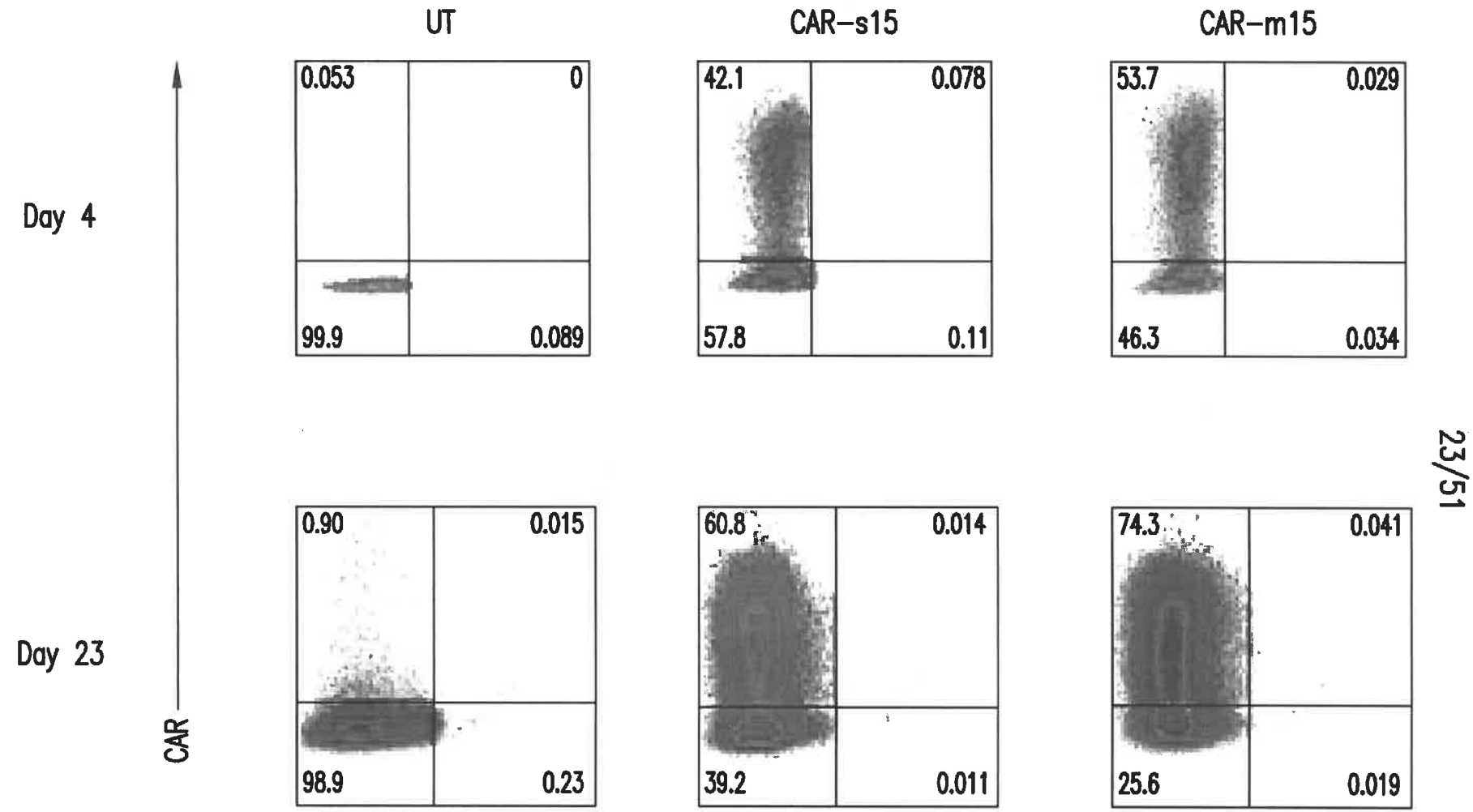

UT
CAR-s15
CAR-m15
Day 4
Day 23
CAR
0.053
0
99.9
0.089
42.1
0.078
57.8
0.11
53.7
0.029
46.3
0.034
0.90
0.015
98.9
0.23
60.8
0.014
39.2
0.011
74.3
0.041
25.6
0.019
Open Channel

FIG.9C

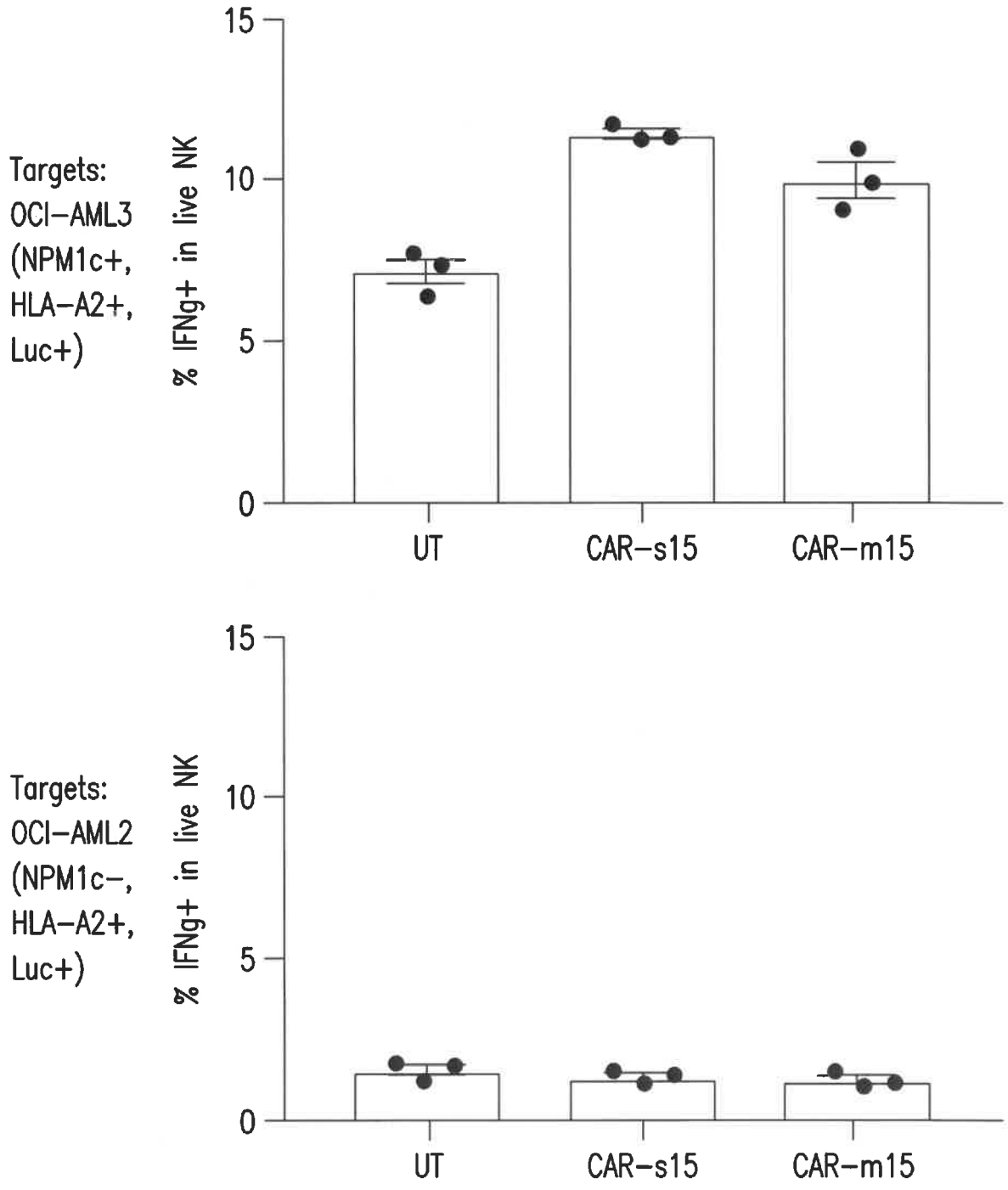

Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15
Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15

FIG.10A

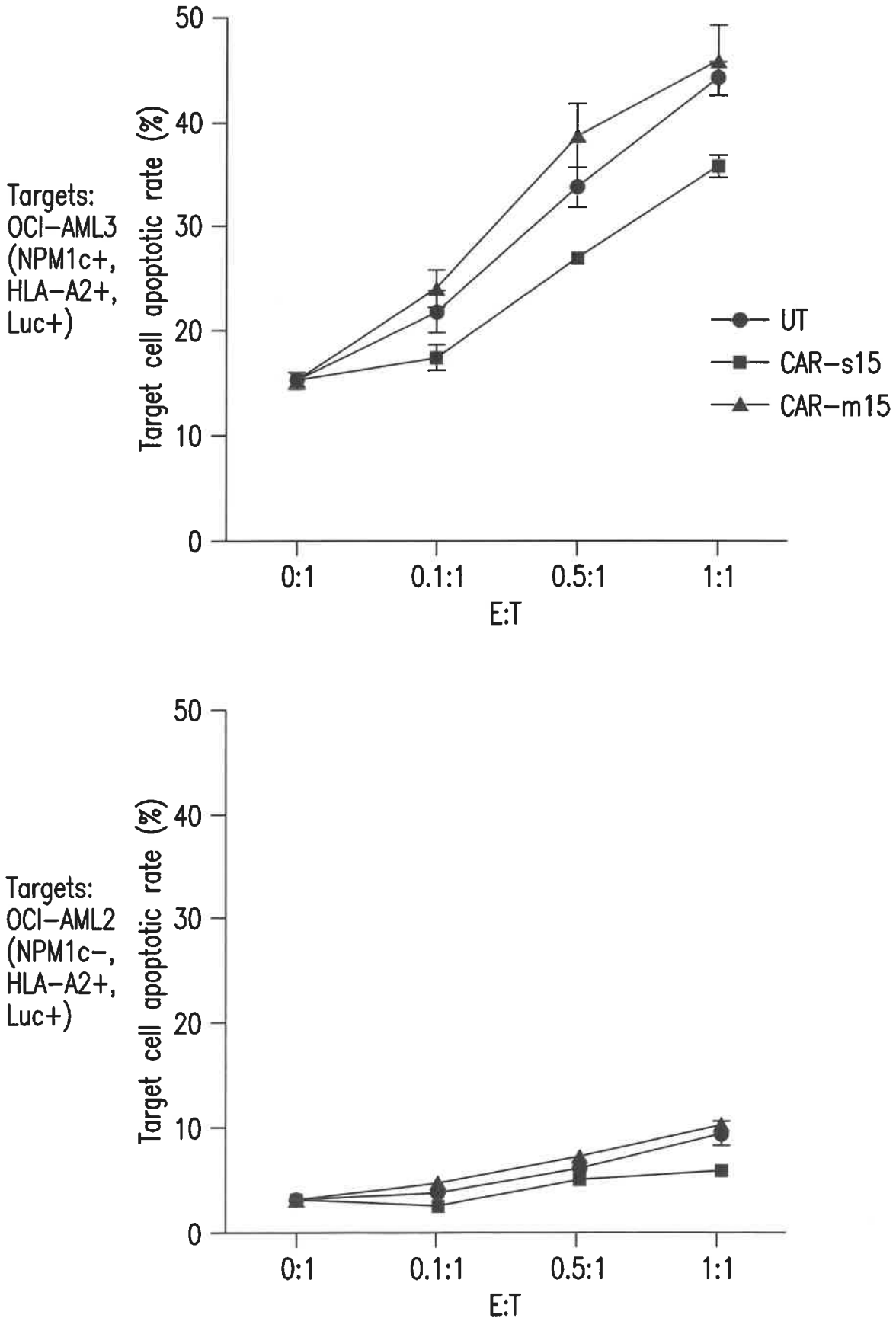

Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR-s15
CAR-m15
Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T

FIG. 10B

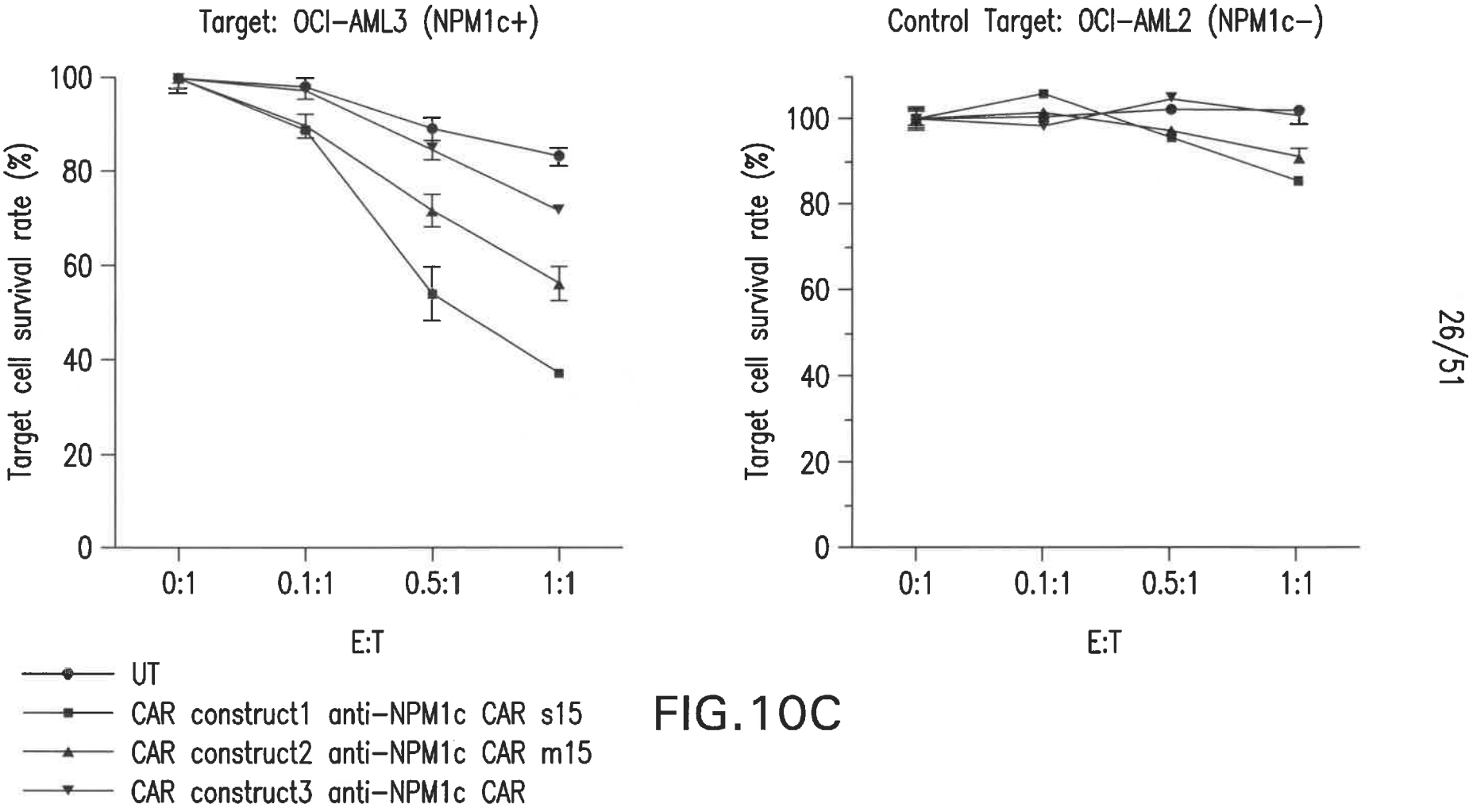

Target: OCI-AML3 (NPM1c+)
Control Target: OCI-AML2 (NPM1c-)
Target cell survival rate (%)
100
80
60
40
20
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR construct1 anti-NPM1c CAR s15
CAR construct2 anti-NPM1c CAR m15
CAR construct3 anti-NPM1c CAR

FIG.10C

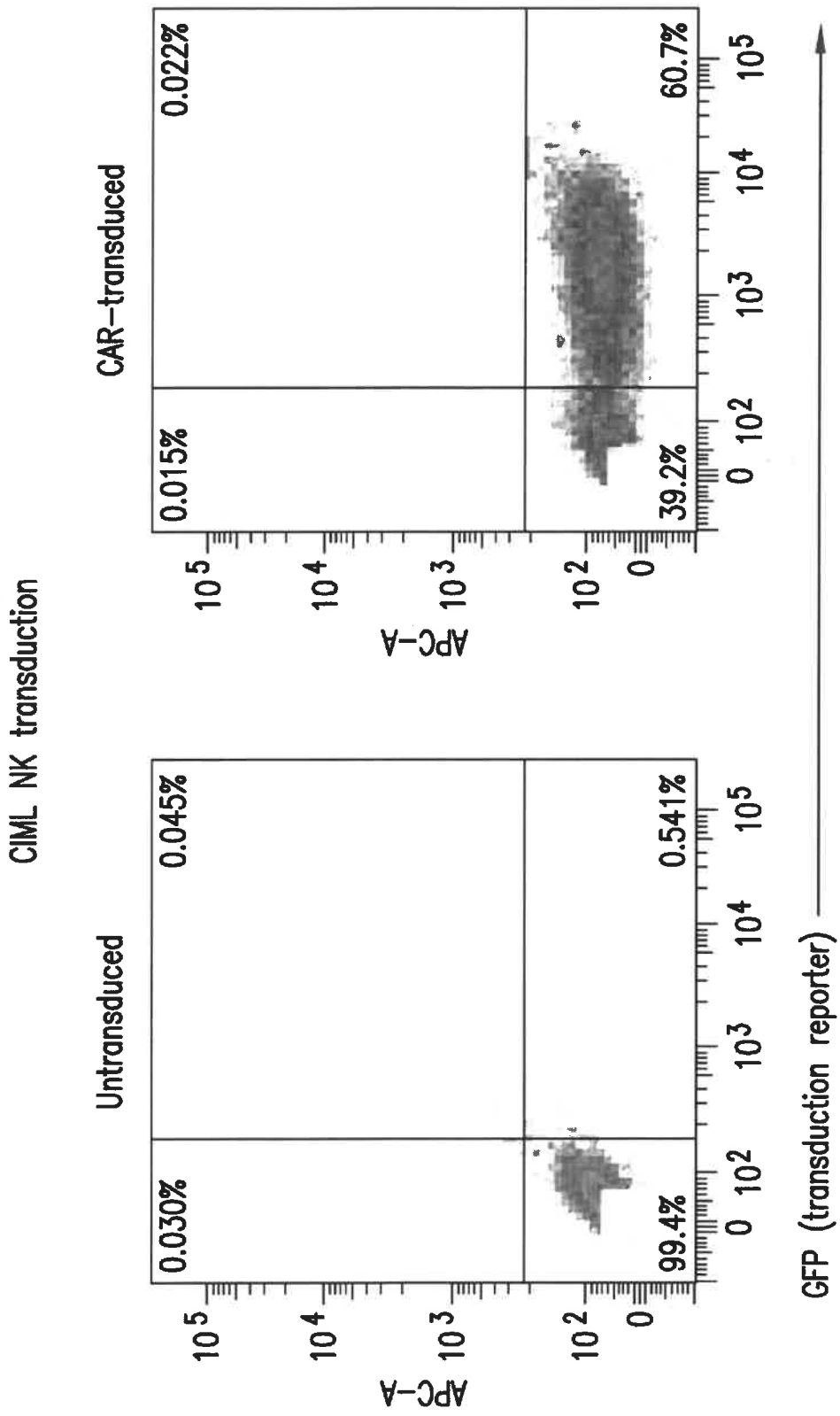

CIML NK transduction
Untransduced
CAR−transduced
0.030%
0.045%
99.4%
0.541%
0.015%
0.022%
39.2%
60.7%
APC−A
GFP (transduction reporter)
0
10²
10³
10⁴
10⁵

FIG.10D

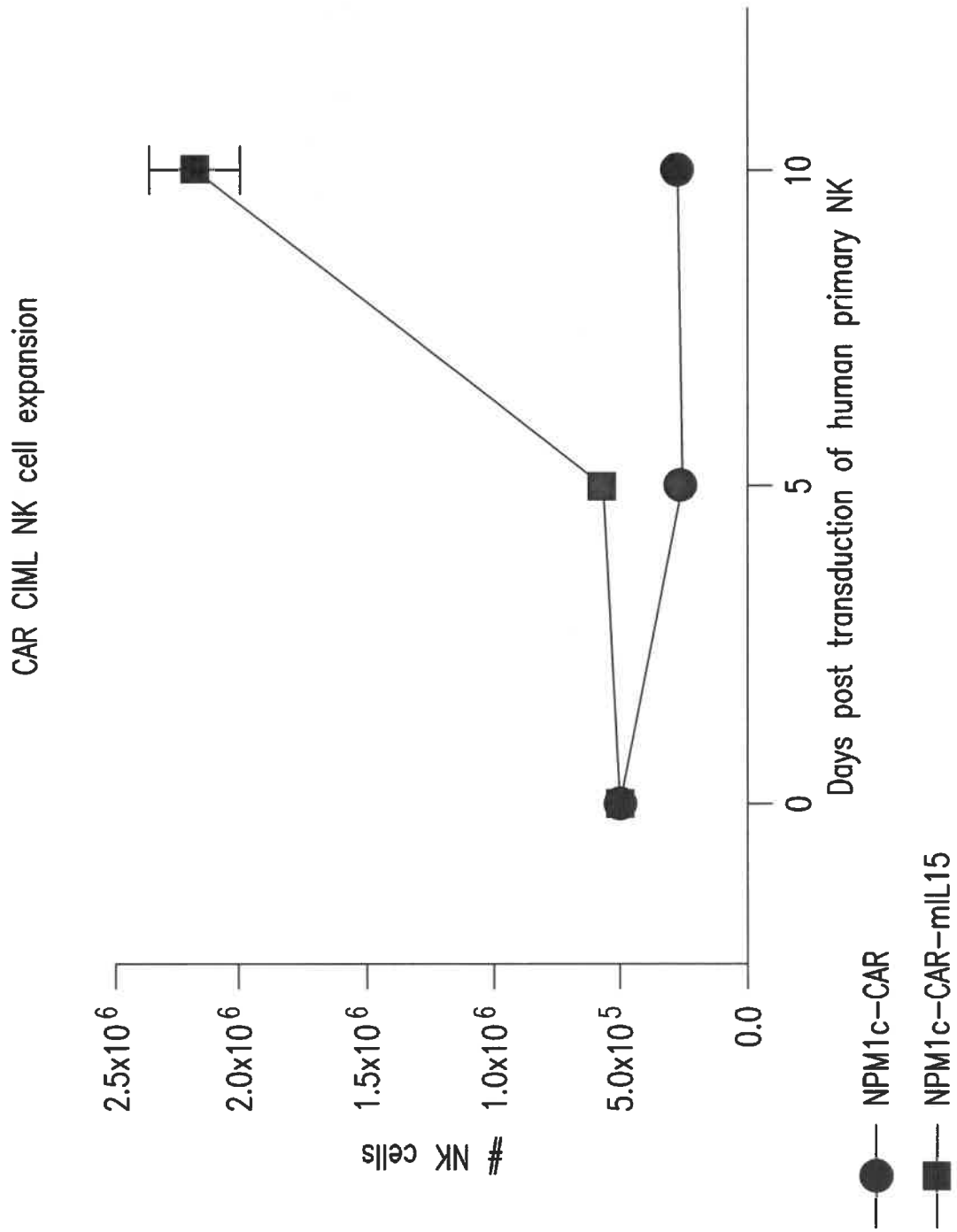

CAR CIML NK cell expansion
NK cells
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
5
10
Days post transduction of human primary NK
NPM1c-CAR
NPM1c-CAR-mIL15

FIG. 10E

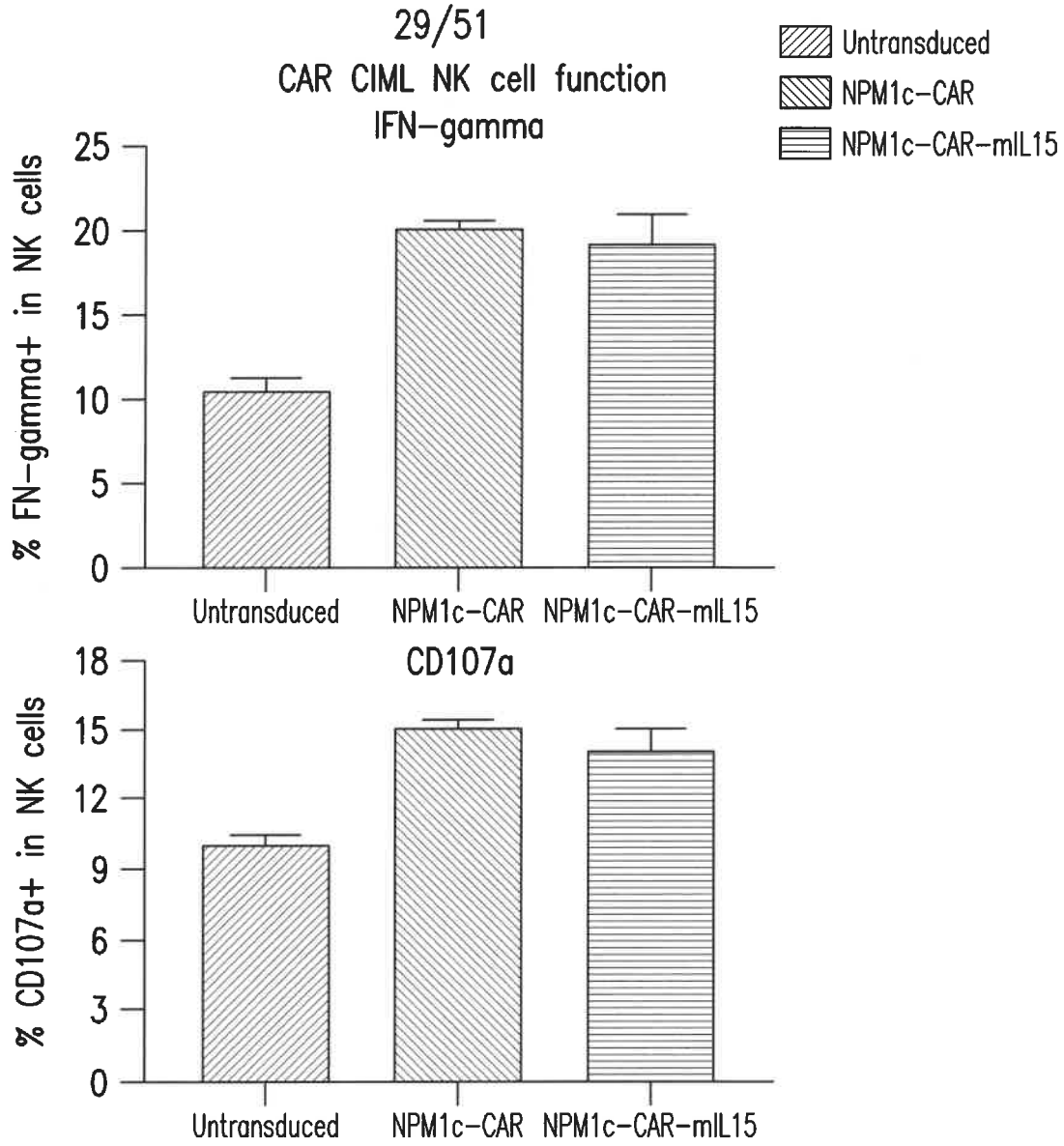


FIG.10F

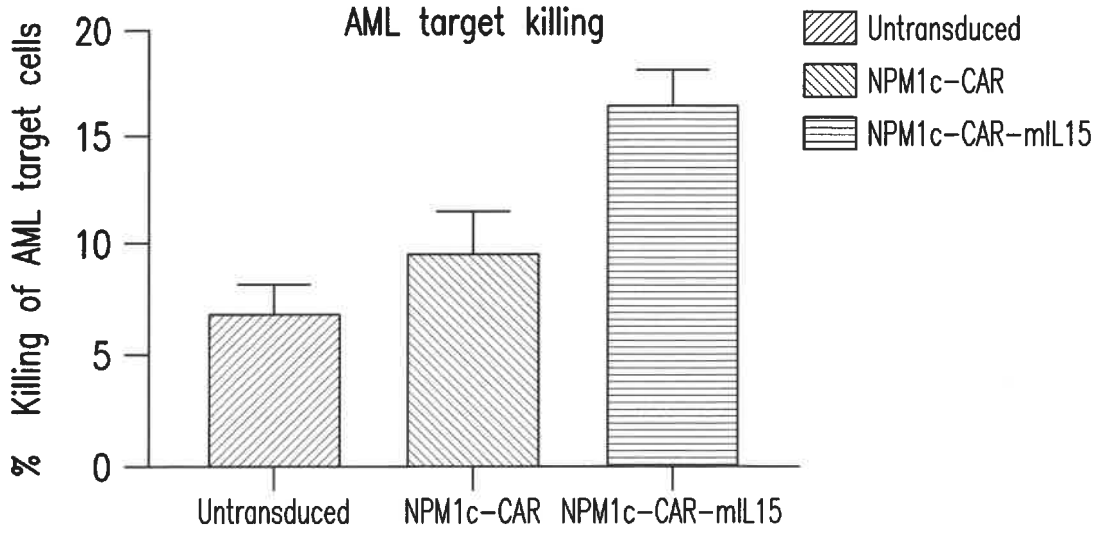


FIG.10G

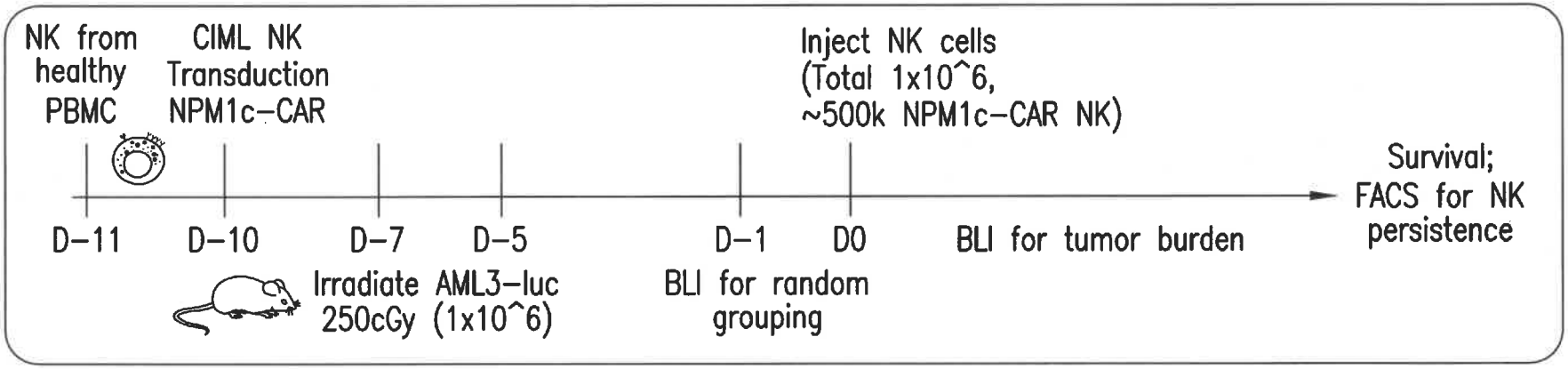

NK from healthy PBMC
CIML NK Transduction NPM1c-CAR
Inject NK cells (Total 1x10^6, ~500k NPM1c-CAR NK)
Survival; FACS for NK persistence
D-11
D-10
D-7
D-5
D-1
D0
BLI for tumor burden
Irradiate 250cGy
AML3-luc (1x10^6)
BLI for random grouping

FIG.11A

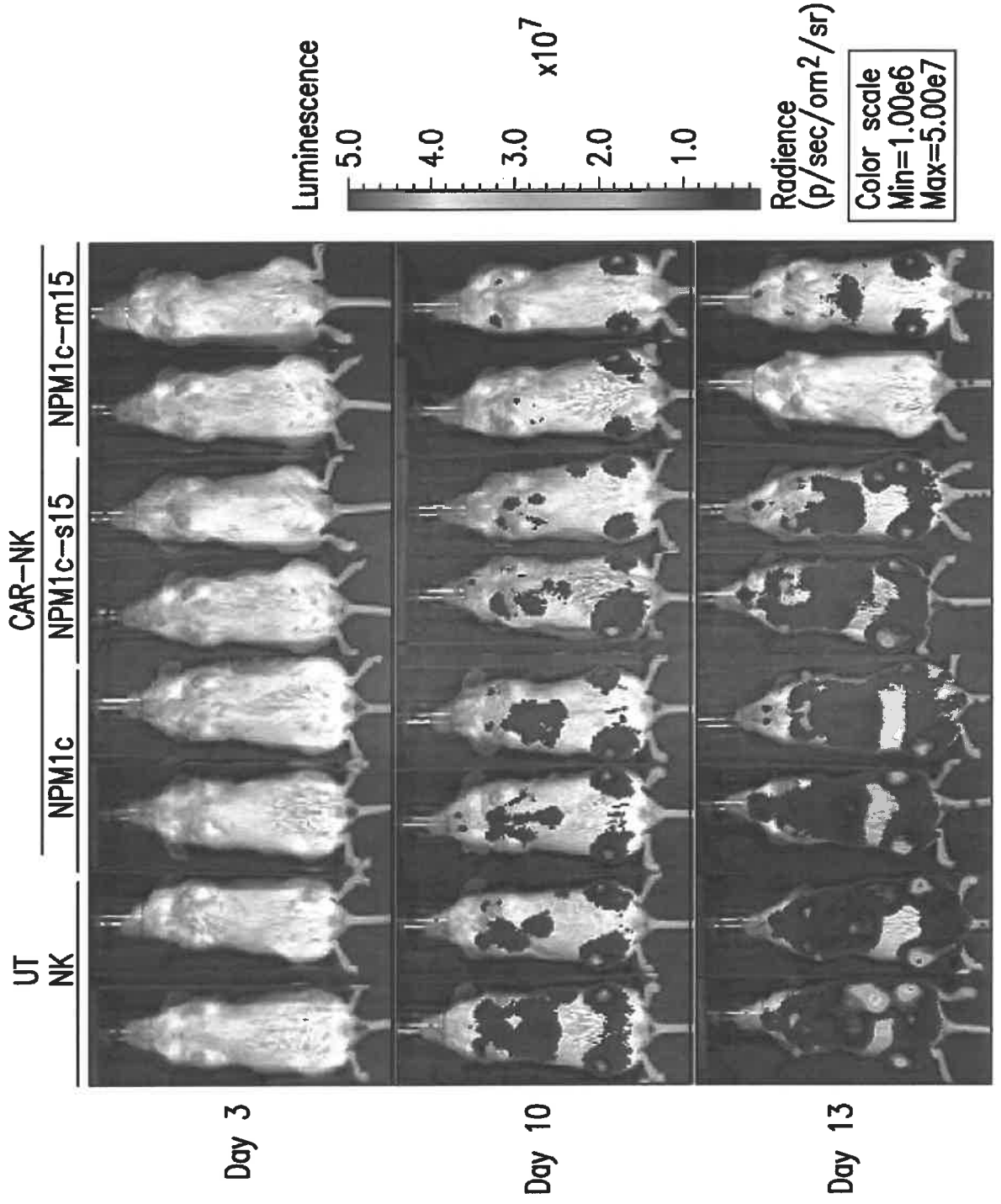

UT
NK
CAR-NK
NPM1c
NPM1c-s15
NPM1c-m15
Day 3
Day 10
Day 13
Luminescence
5.0
4.0
3.0
2.0
1.0
x10^7
Radience (p/sec/cm^2/sr)
Color scale
Min=1.00e6
Max=5.00e7

FIG. 11B

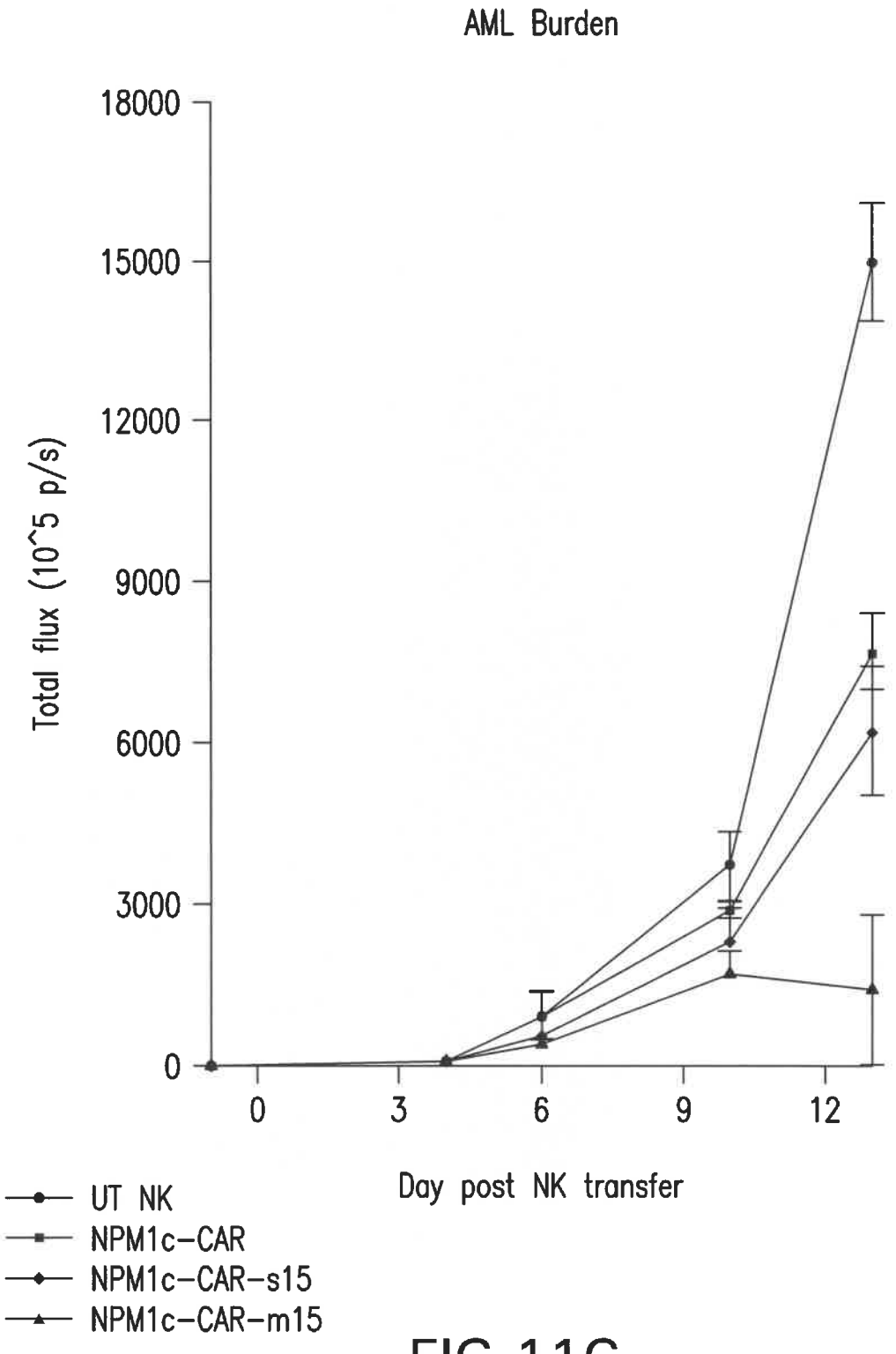

AML Burden
18000
15000
12000
9000
6000
3000
0
Total flux (10^5 p/s)
0
3
6
9
12
Day post NK transfer
UT NK
NPM1c-CAR
NPM1c-CAR-s15
NPM1c-CAR-m15

FIG.11C

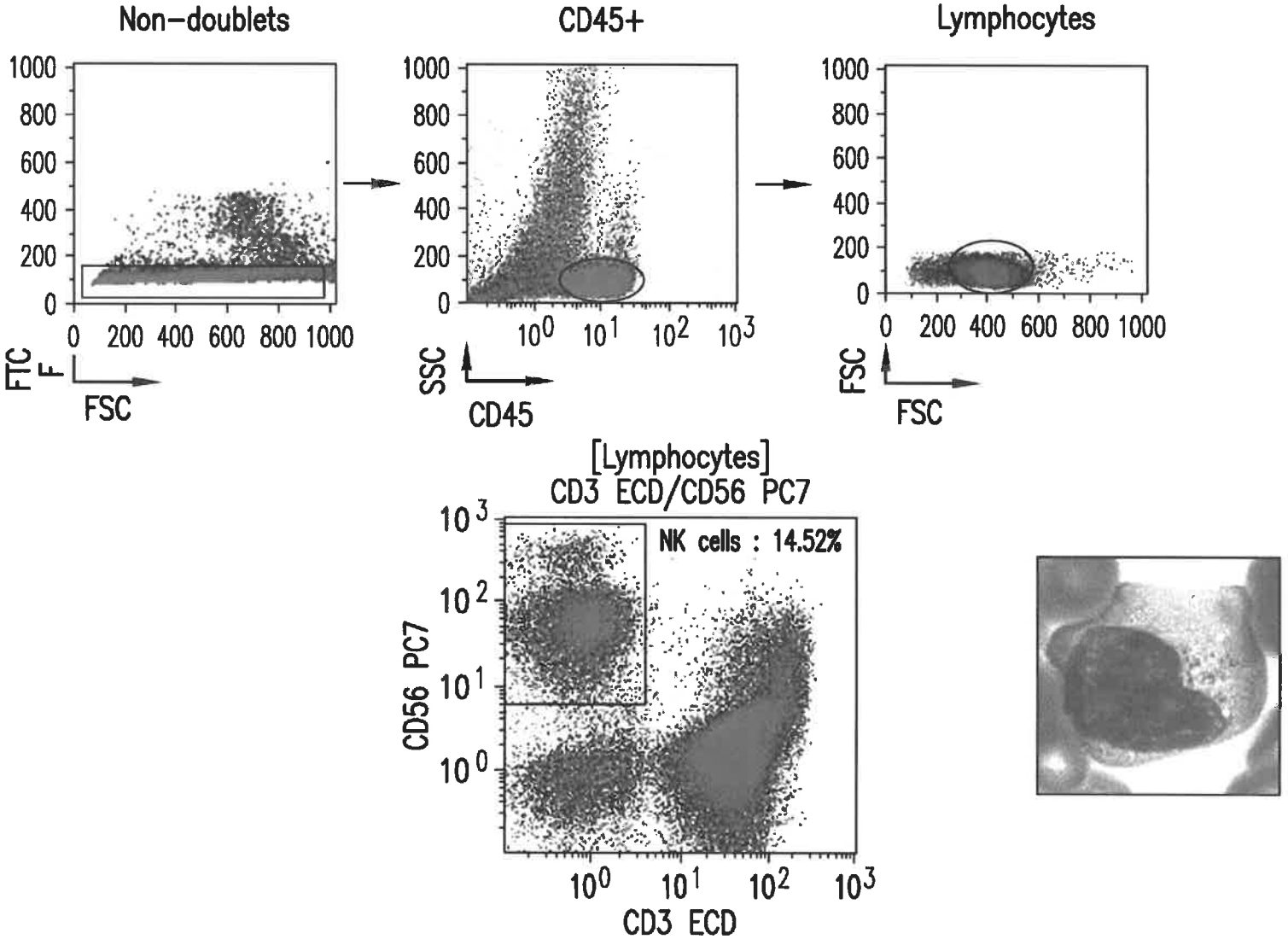

Non−doublets
CD45+
Lymphocytes
1000
800
600
400
200
0
0 200 400 600 800 1000
FTC F
FSC
10^0 10^1 10^2 10^3
SSC
CD45
FSC
[Lymphocytes]
CD3 ECD/CD56 PC7
NK cells : 14.52%
CD56 PC7
CD3 ECD

FIG.12

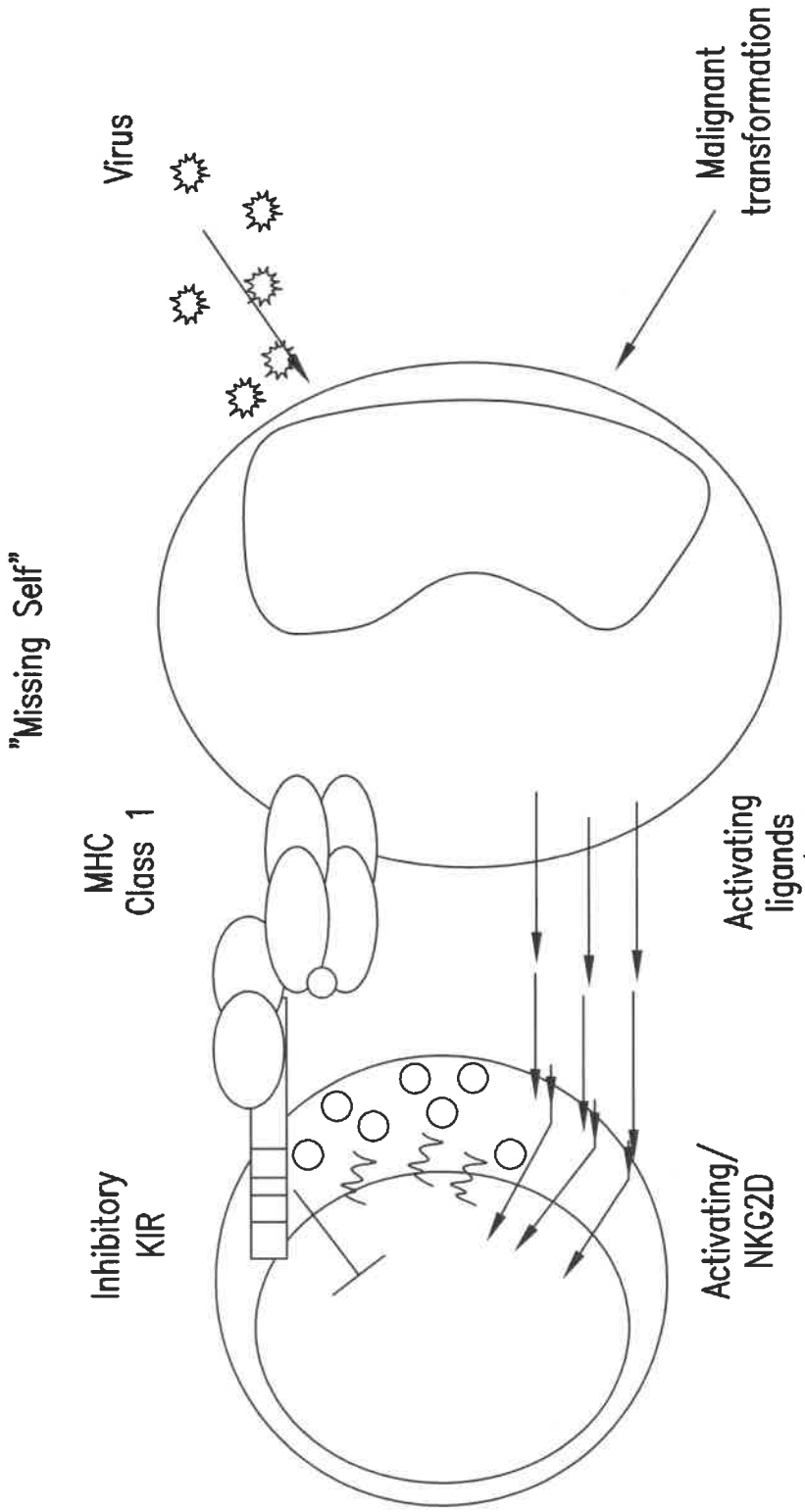
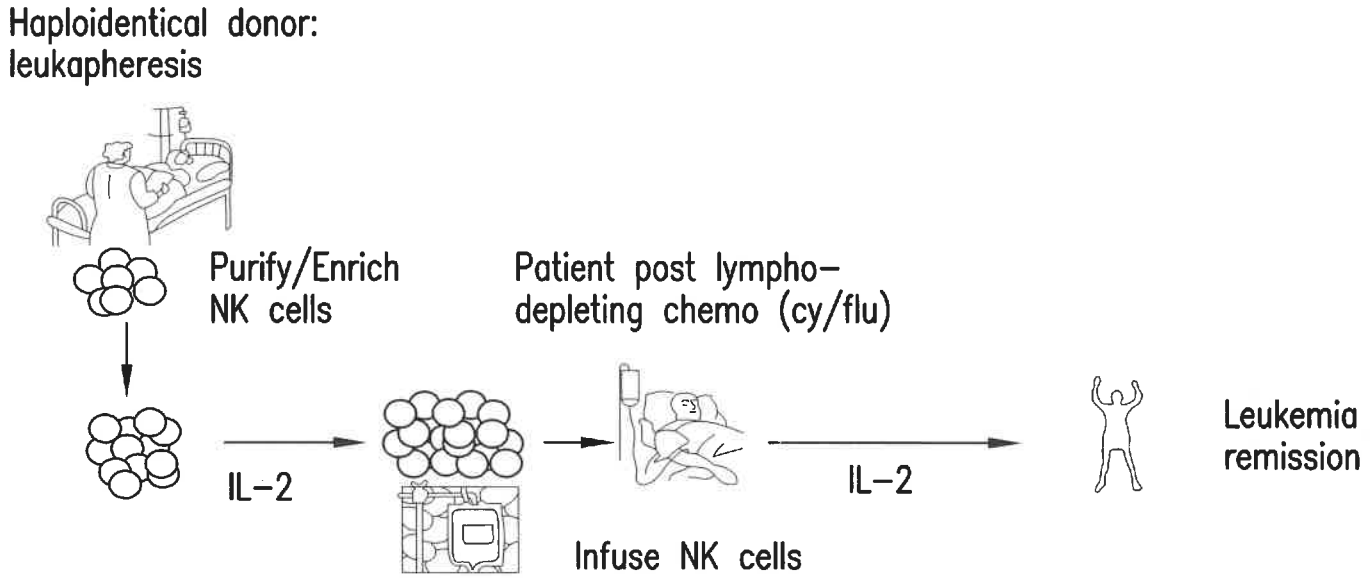

Virus
Malignant transformation
"Missing Self"
MHC Class 1
Activating ligands MIC-A/MIC-B
Inhibitory KIR
Activating/ NKG2D

FIG.13



Conventional NK cells have a life span of around 12-14 days
Limited expansion and persistence *in vivo*
Modest success mostly restricted to AML and MDS patients (~5-25%)
Primary NK cells are resistant to genetic manipulation

FIG.14

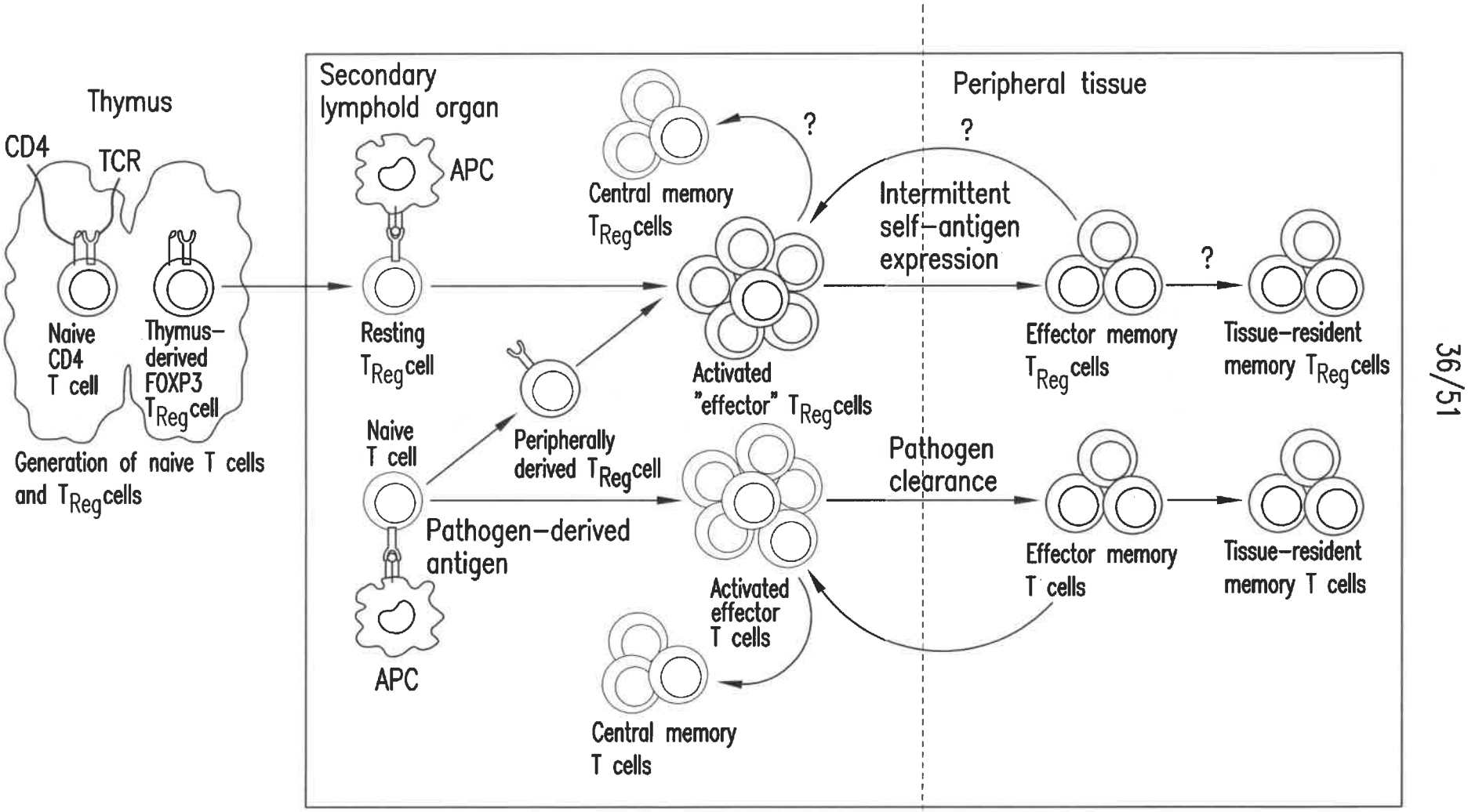

Thymus
CD4
TCR
Naive CD4 T cell
Thymus-derived FOXP3 T_{Reg} cell
Generation of naive T cells and T_{Reg} cells
Secondary lymphoid organ
APC
Resting T_{Reg} cell
Central memory T_{Reg} cells
?
Activated "effector" T_{Reg} cells
Peripherally derived T_{Reg} cell
Naive T cell
Pathogen-derived antigen
APC
Activated effector T cells
Central memory T cells
Peripheral tissue
?
Intermittent self-antigen expression
Effector memory T_{Reg} cells
?
Tissue-resident memory T_{Reg} cells
Pathogen clearance
Effector memory T cells
Tissue-resident memory T cells

FIG.15

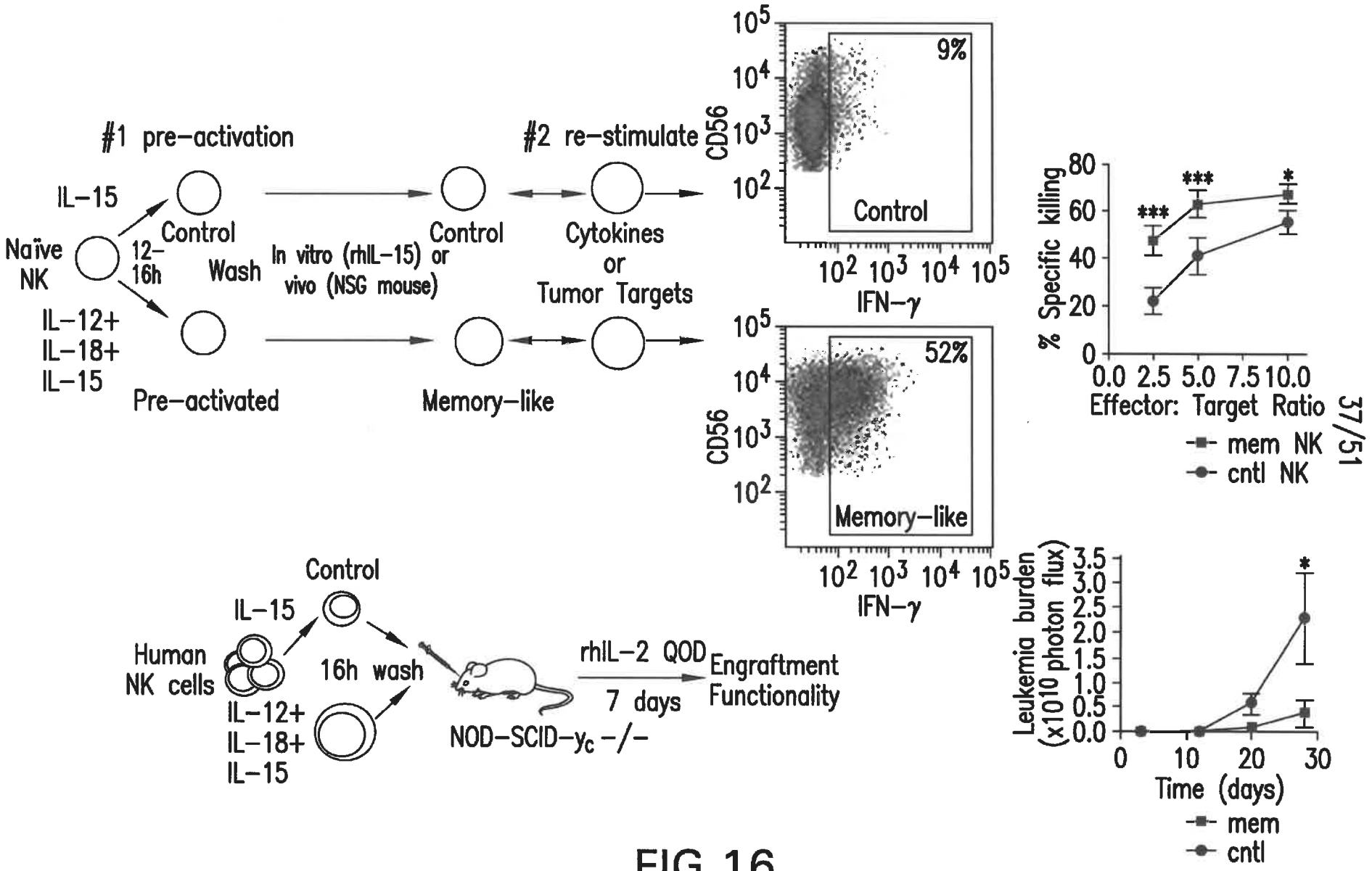
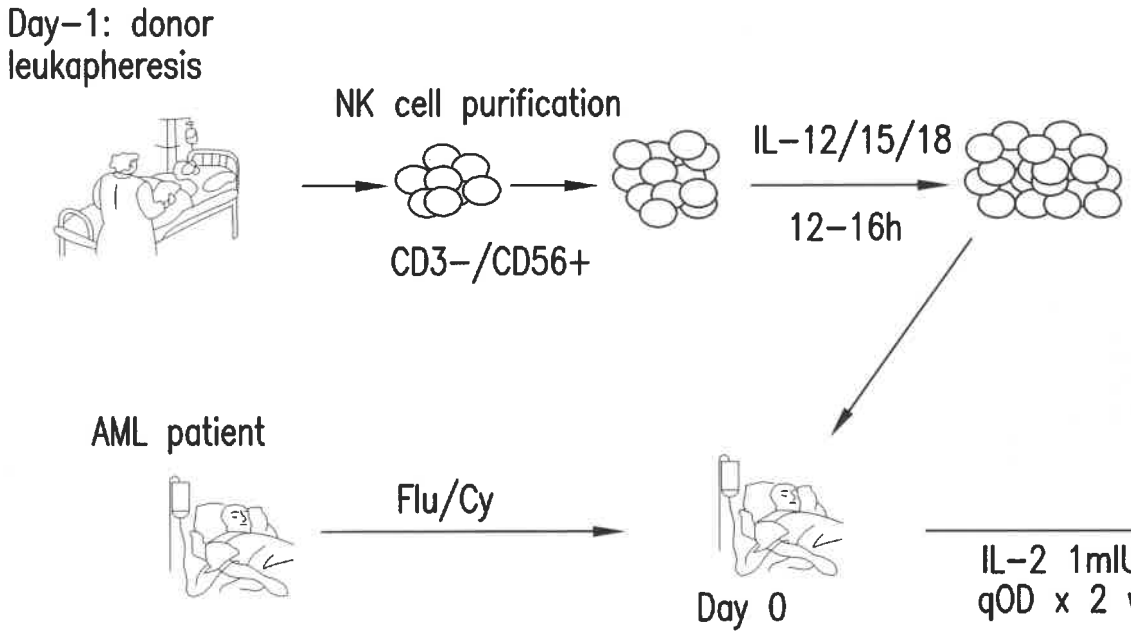

#1 pre-activation
#2 re-stimulate
IL-15
Control
Naïve NK
12-16h
Wash
In vitro (rhIL-15) or vivo (NSG mouse)
Control
Cytokines or Tumor Targets
IL-12+ IL-18+ IL-15
Pre-activated
Memory-like
CD56
IFN-γ
9%
Control
52%
Memory-like
% Specific killing
Effector: Target Ratio
mem NK
cntl NK
Control
IL-15
Human NK cells
16h wash
IL-12+ IL-18+ IL-15
NOD-SCID-γc -/-
rhIL-2 QOD
7 days
Engraftment Functionality
Leukemia burden (x1010 photon flux)
Time (days)
mem
cntl

FIG. 16



Cell dose levels:

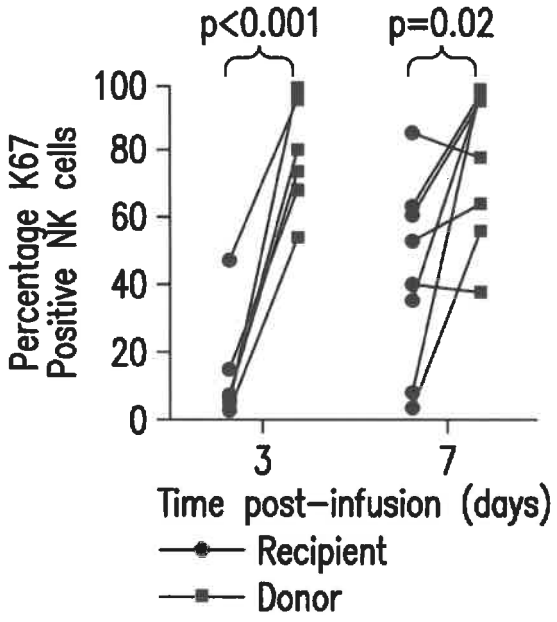
1. 0.5 x 10^6/kg
2. 1.0 x 10^6/kg
3. Max capped at $10x10^6$/kg

Endpoints:

Primary:
Safety/MTD

Secondary:
Leukemia Response
In vivo biology

FIG.17



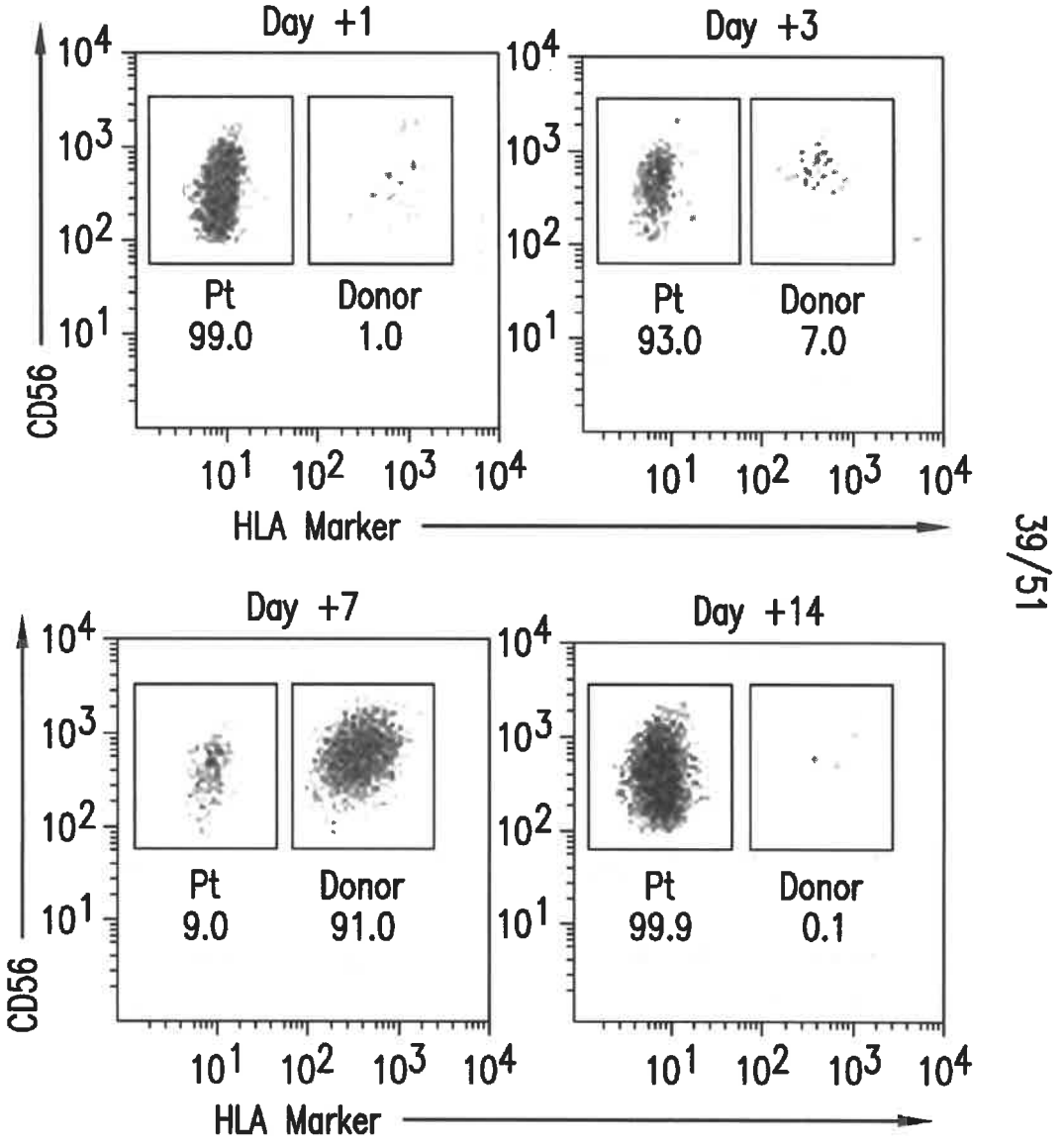


FIG.18
CONTINUED

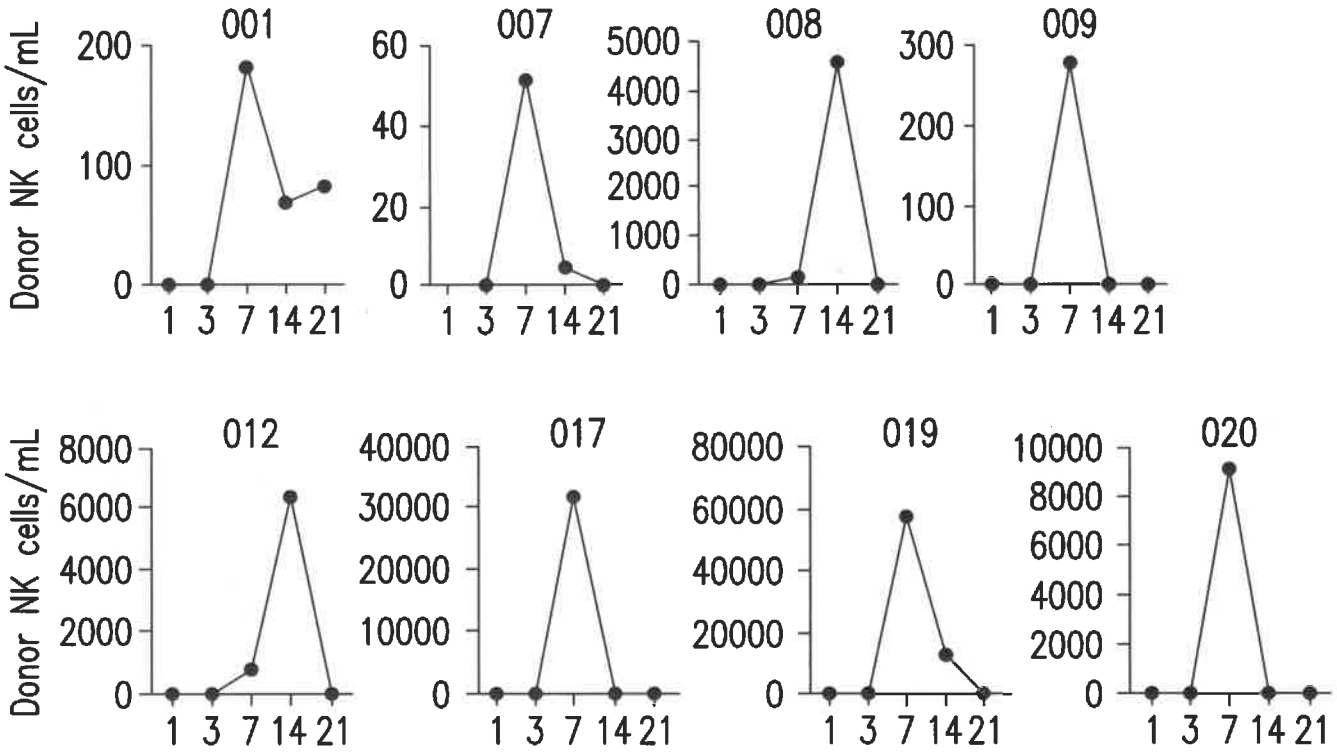


FIG. 18
CONTINUED

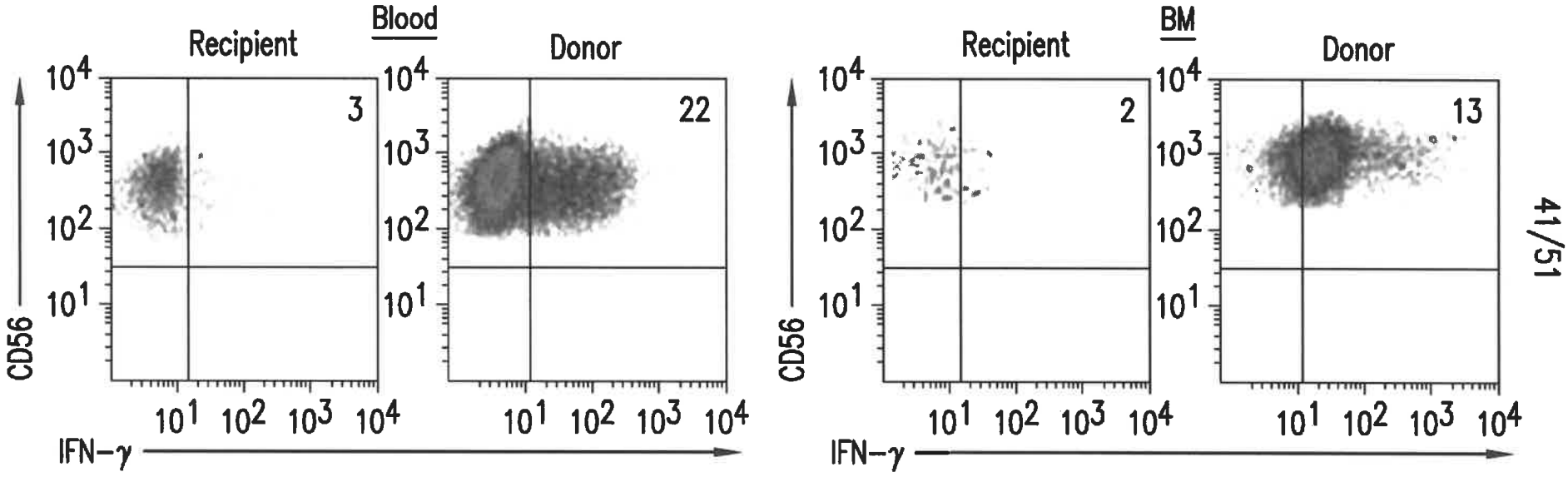


FIG.18
CONTINUED

UPN	Dose Level	Gender	Age (years)	WHO Dx	BM Blast (%)	KIR Ligand Mismatch	IWG Response	DLT	GVHD
001	1	M	73	M2	16	Yes	TF-PD	No	No
006	1	M	70	M0	28	Yes	TF-PD	No	No
007	1	M	77	M0	47	Yes	CR	No	No
008	2	M	76	t-AML	17	Yes	TF-PD	No	No
009	2	F	73	M1	80	No	MLFS	No	No
012	2	F	71	M5	15	Yes	CR	No	No
017	3	M	64	t-AML	69	Yes	TF-PD	No	No
019	3	F	71	M5	15	Yes	CR	No	No
020	3	M	60	MDS-AML	13	Yes	CRp	No	No
023	3	M	83	M1	86	No	CRp	No	No
025	3	M	72	t-AML	1	No	CR	No	No
027	3	M	76	M1	In CR	No	CRp	No	No
028	3	F	69	HMA failed MDS	6	No	Marrow CR	No	No

>50% CR Rate

2 Long term survivors

1 Died in CR

FIG.19

Relapse remains most common cause of death.

Donor lymphocyte infusion (DLI) with suboptimal outcomes (15–20% response)

Potential for more GVHD due to HLA mismatch

Unmet need

Phase 1 a/b study in relapsed patients

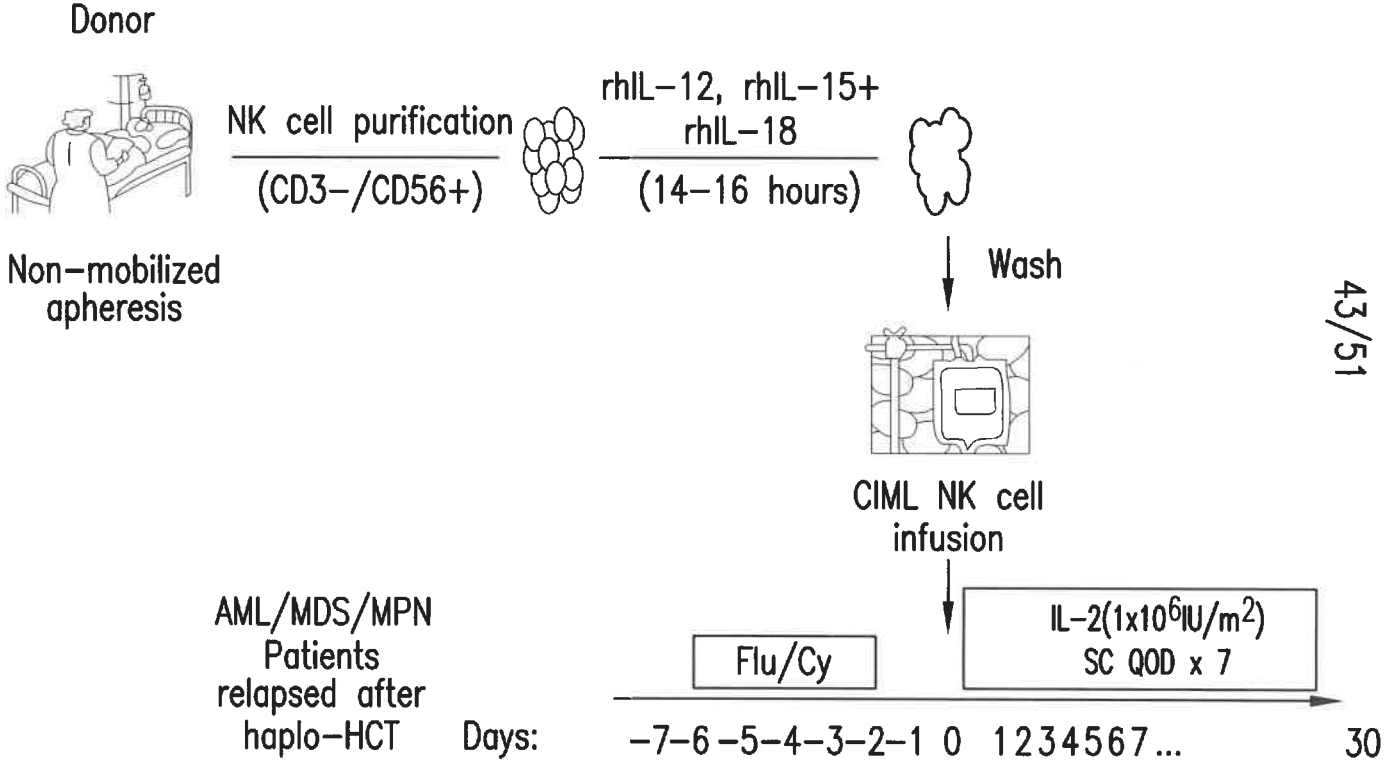


FIG.20

First 4 patients cleared disease, 2nd patient *(TP53 mutated)* MRD– by flow & RHP

Massive expansion & prolonged persistence

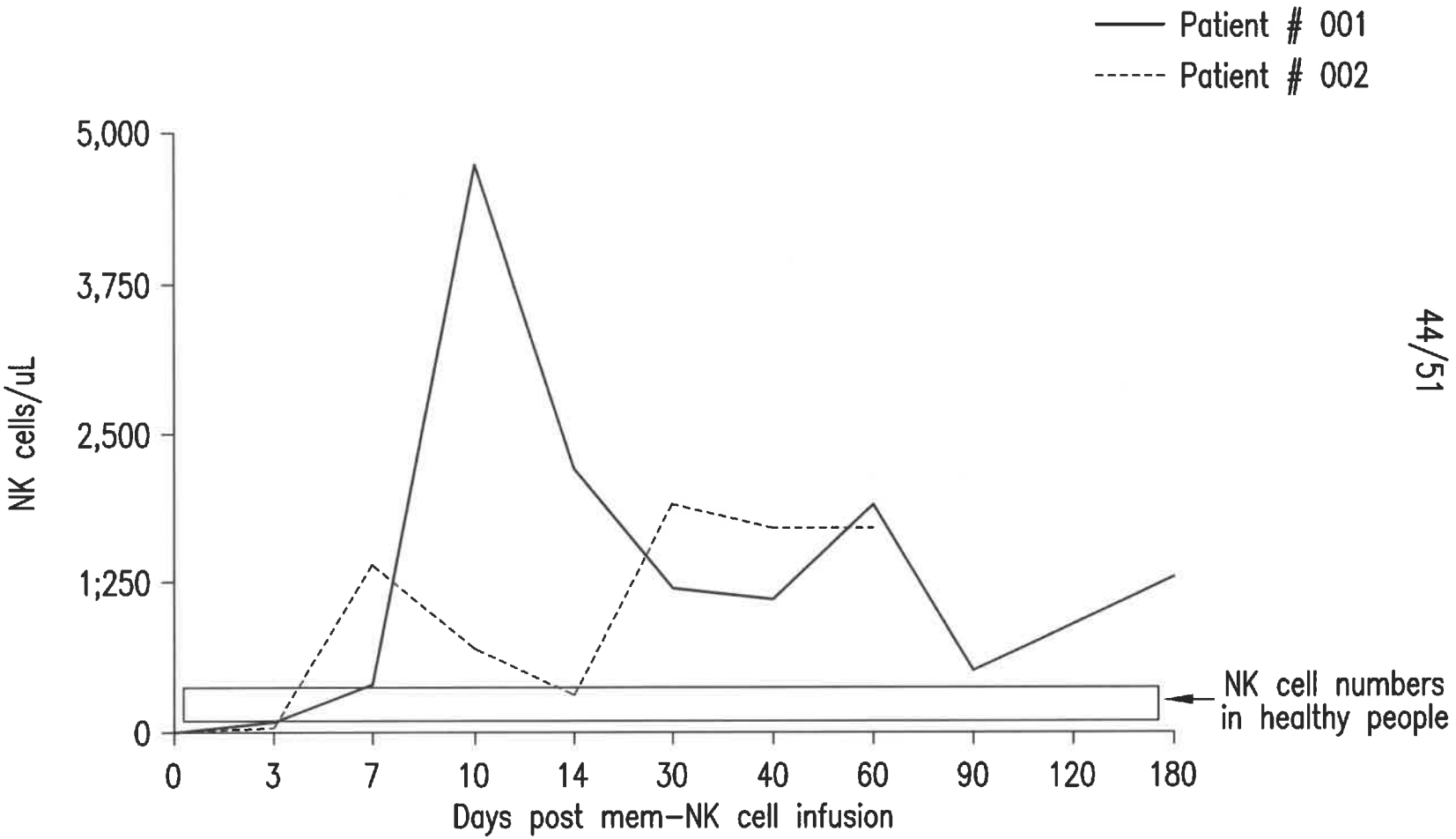


FIG.21

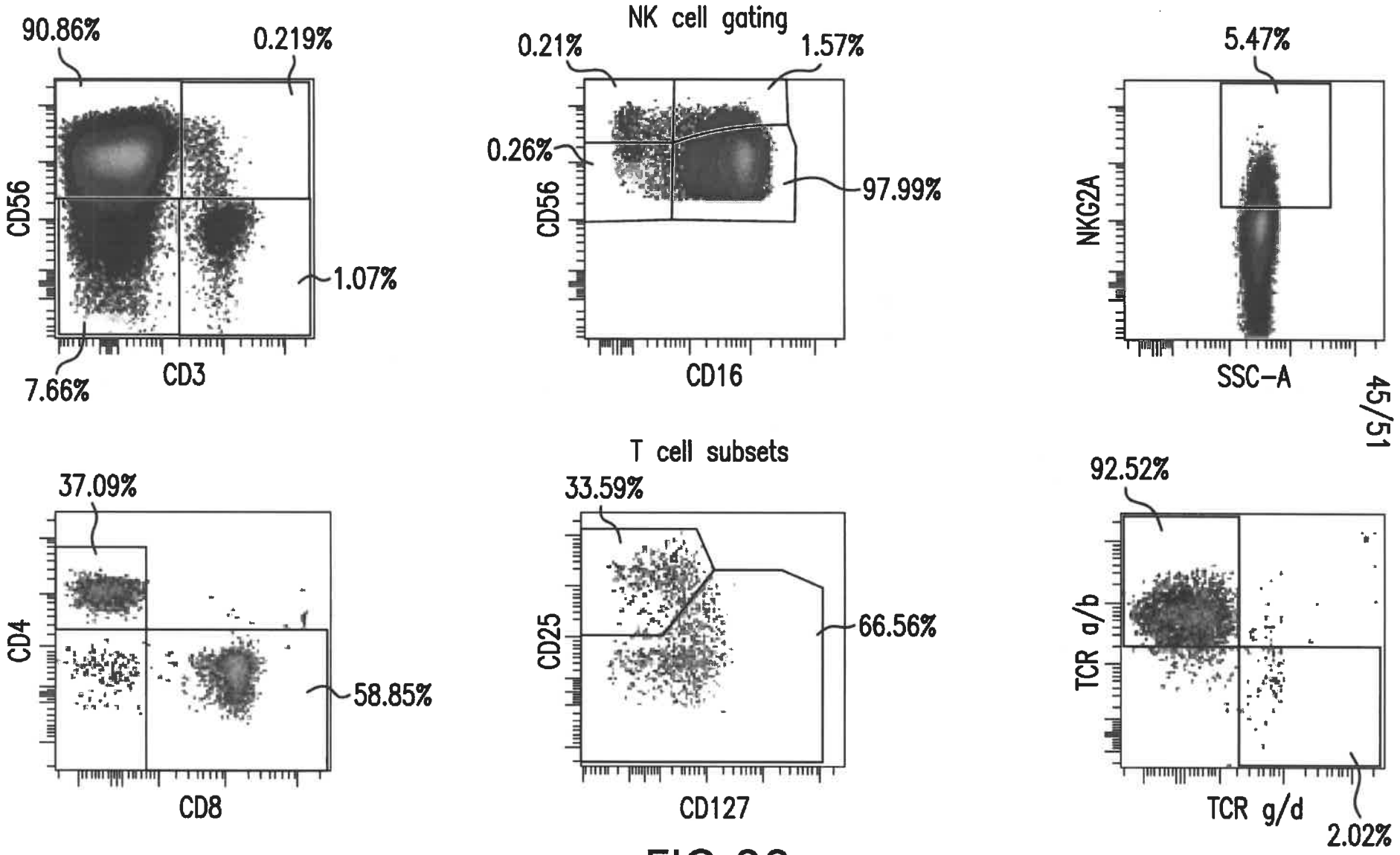
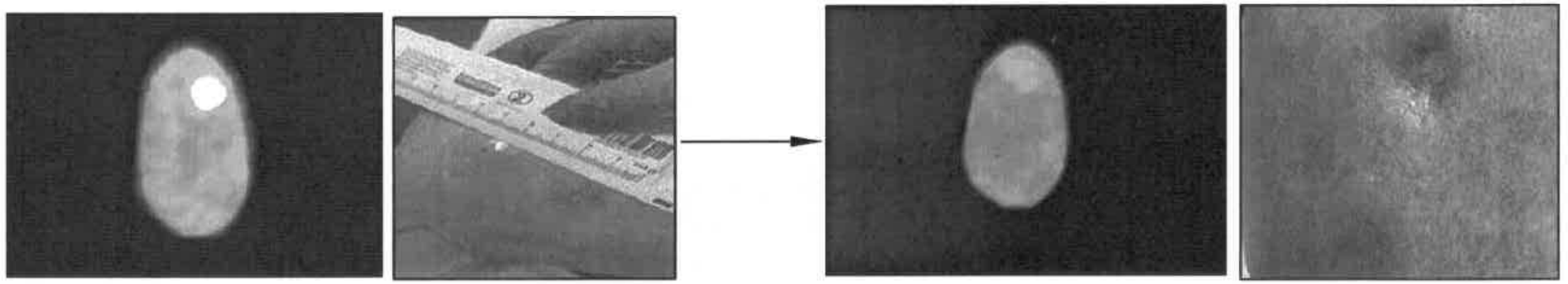

90.86%
0.219%
CD56
1.07%
7.66%
CD3
NK cell gating
0.21%
1.57%
0.26%
97.99%
CD56
CD16
5.47%
NKG2A
SSC-A
45/51
37.09%
CD4
58.85%
CD8
T cell subsets
33.59%
CD25
66.56%
CD127
92.52%
TCR a/b
TCR g/d
2.02%

FIG.22

66M, diagnosed in 5/2019, achieves CR with tagraxofusp (SL-401)
Underwent RIC Haplo HCT on 9/2019 but relapsed 5 months after Haplo HCT

Underwent memory-like NK cell immunotherapy in 8/2020 In CR!



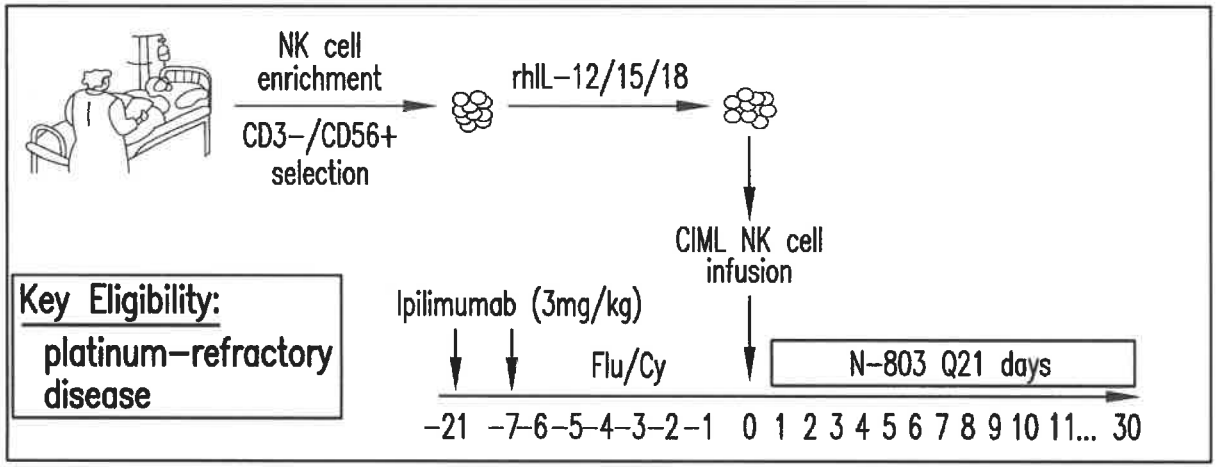
Pre-NK cell infusion Day 30 post NK cell infusion

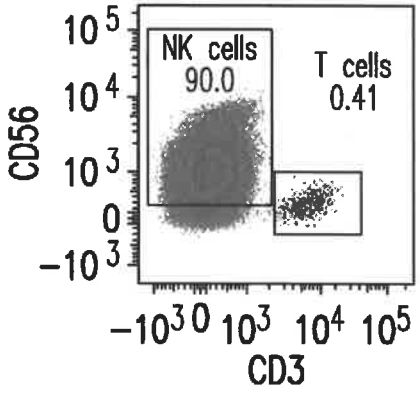
Mature NK cells constituted 75% of the 1350/uL peripheral blood lymphocytes at day +28 after infusion

In vitro BPDCN targeting with ML-NK cells

FIG.23

Anti-CTLA-4 antibody leads to Treg depletion to facilitate NK cell activity
NK cell infiltration associated with favorable outcomes in H&N ca patients
N-803 is an IL-15 super-agonist which enhances NK cell activation in vivo





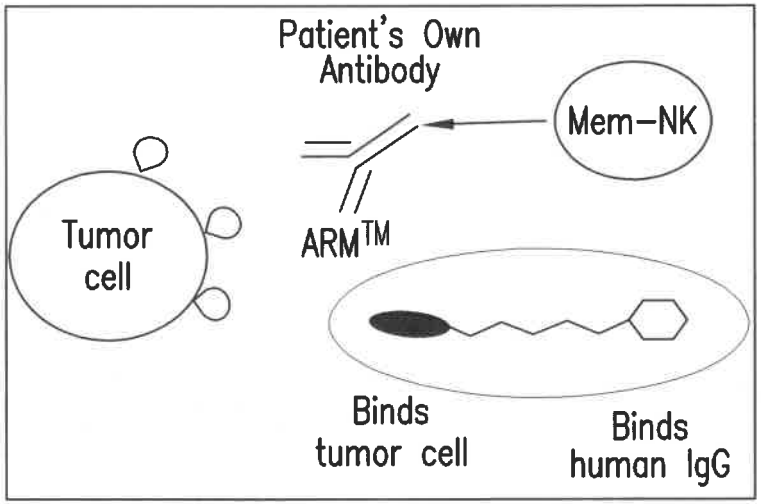
First patient treated on 9/15 (platinum/cetux/pembro failure)

>2500 mature NK cells/uL on day 21

IL-15 injection site rash



FIG.24


Patient's Own
Antibody
Mem–NK
Tumor
cell
ARM™
Binds
tumor cell
Binds
human IgG

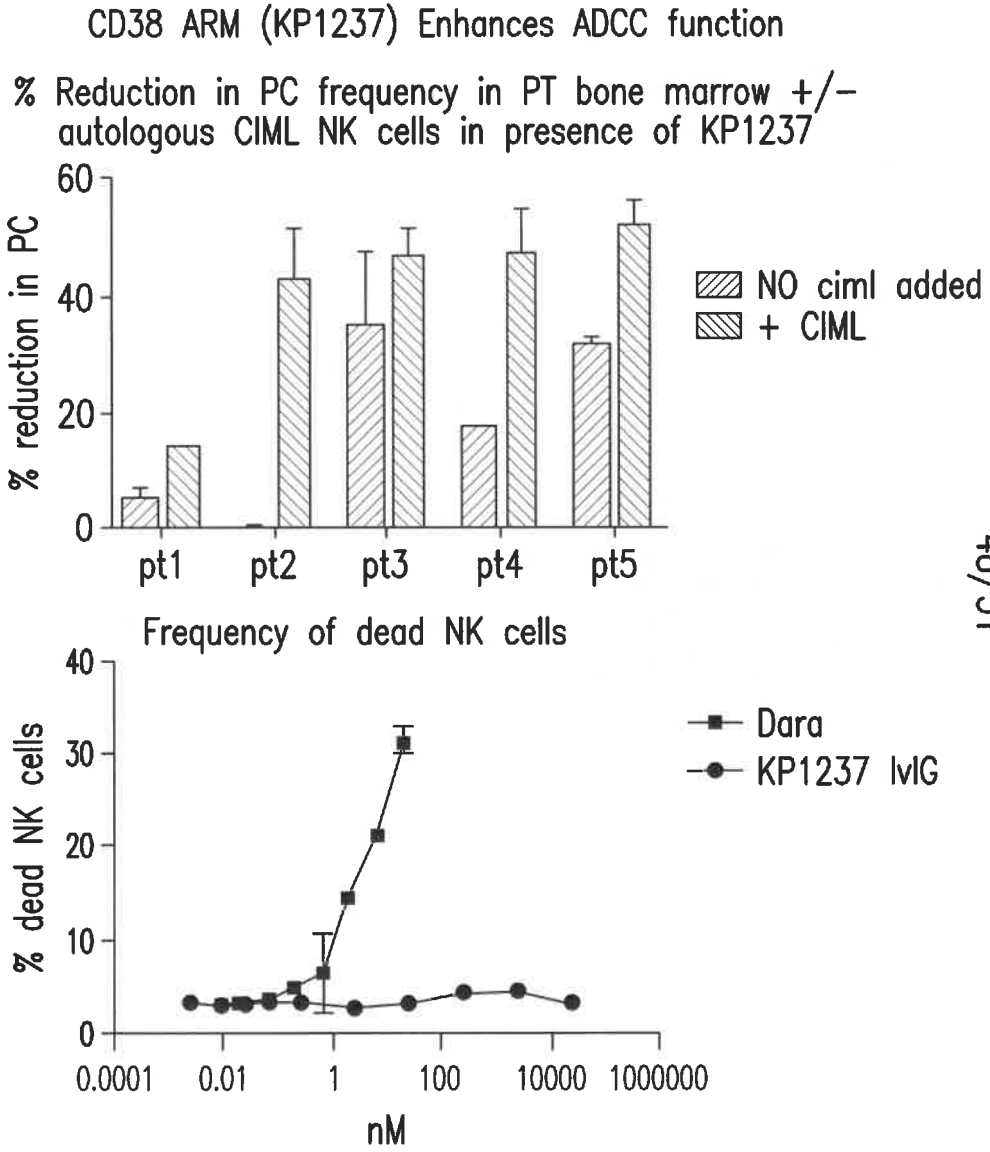

CD38 ARM (KP1237) Enhances ADCC function
% Reduction in PC frequency in PT bone marrow +/- autologous CIML NK cells in presence of KP1237
% reduction in PC
60
40
20
0
pt1
pt2
pt3
pt4
pt5
NO ciml added
+ CIML
Frequency of dead NK cells
% dead NK cells
40
30
20
10
0
0.0001
0.01
1
100
10000
1000000
nM
Dara
KP1237 IvIG

FIG.25

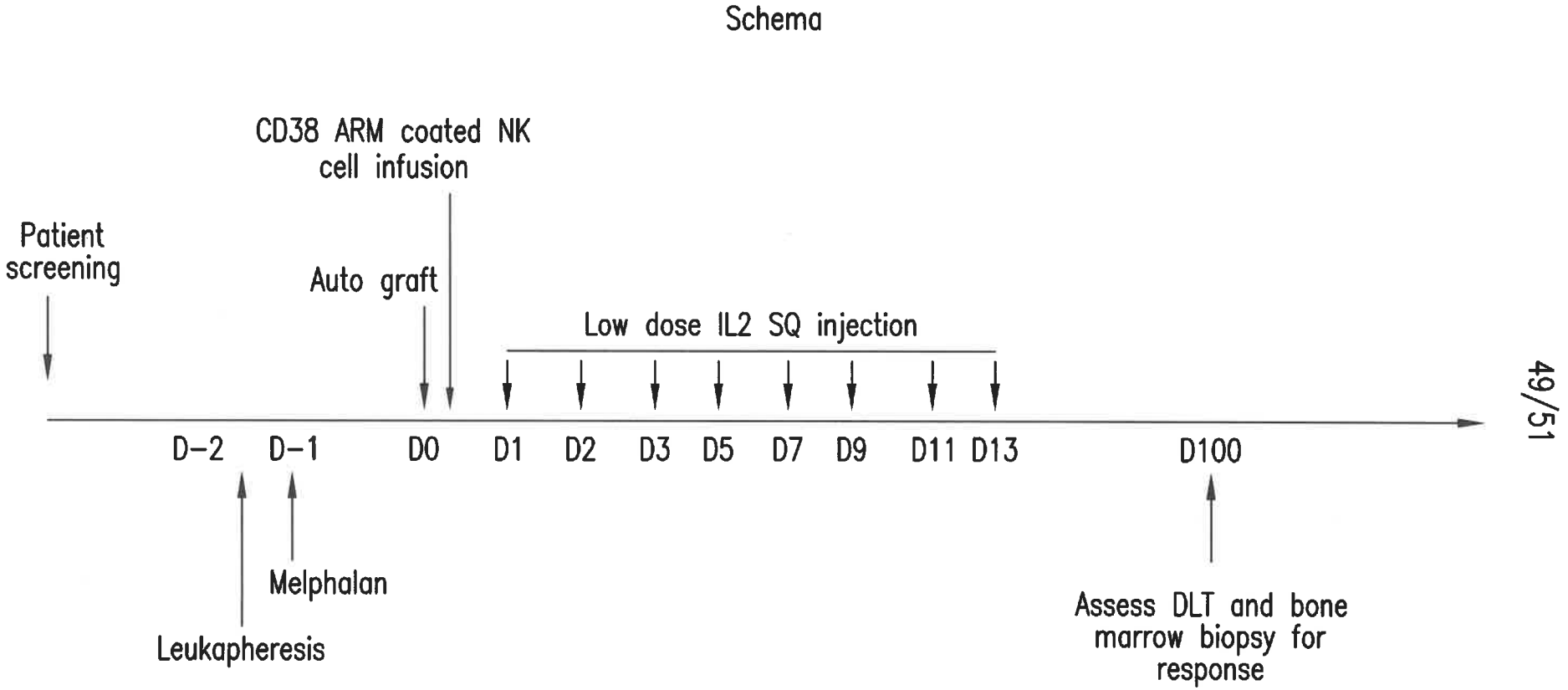

Schema
CD38 ARM coated NK cell infusion
Patient screening
Auto graft
Low dose IL2 SQ injection
D-2
D-1
D0
D1
D2
D3
D5
D7
D9
D11
D13
D100
Melphalan
Leukapheresis
Assess DLT and bone marrow biopsy for response

FIG.26

NK cell CARs are safe (no CRS/no neurotoxicity), response rate similar to CAR T cells

Limited efficacy of CAR T cells in AML/MDS

Dual targeting of myeloid blasts by Mem-NK cells armed with CARs *(antigen expression triggers CAR vs HLA loss triggers direct NK cell cytotoxicity)*

Primary Peripheral blood NK cells are resistant to transduction

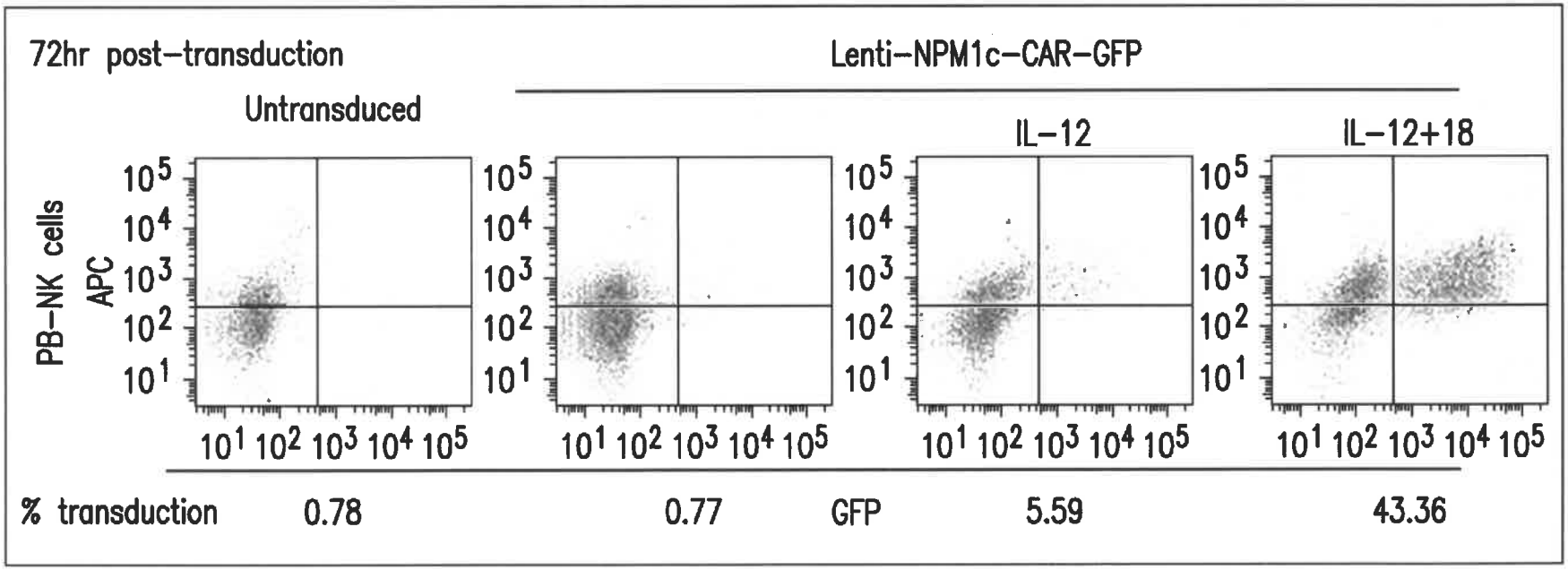


FIG.27

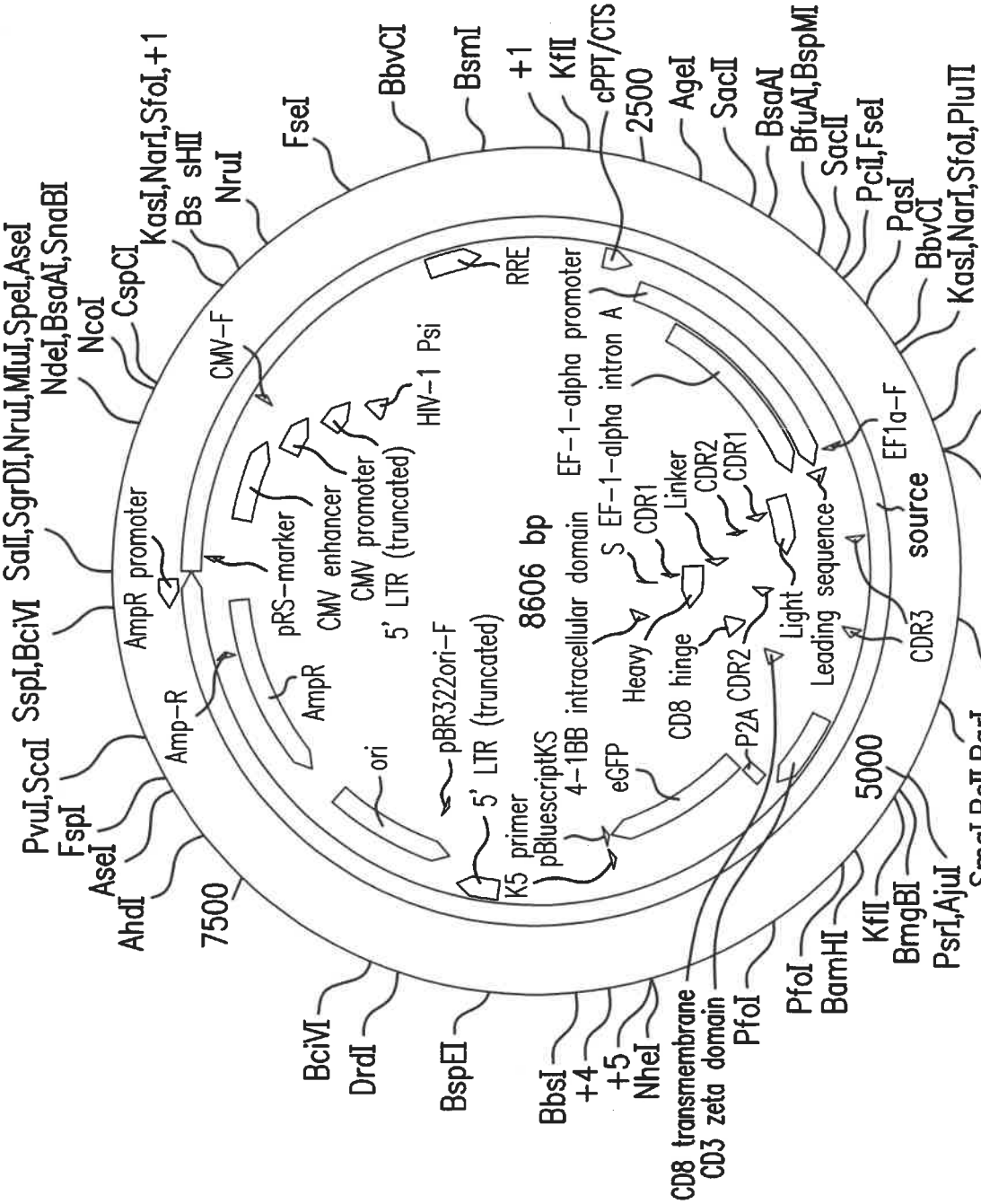

pHIV-CAR-CD19hscFv-P2A-GFP (8606 bp)
8606 bp
5000
7500
AmpR promoter
Amp-R
AmpR
pRS-marker
CMV enhancer
CMV promoter
5' LTR (truncated)
HIV-1 Psi
RRE
CMV-F
ori
pBR322ori-F
5' LTR (truncated)
K5 primer
pBluescriptKS
4-1BB intracellular domain
eGFP
P2A
CD8 hinge
Heavy
S
EF-1-alpha intron A
EF-1-alpha promoter
CDR1
Linker
CDR2
CDR1
Light
Leading sequence
CDR3
CDR2
EF1a-F
source
cPPT/CTS
2500
+1
BsmI
BbvCI
FseI
NruI
Bs sHII
KasI,NarI,SfoI,+1
CspCI
NcoI
NdeI,BsaAI,SnaBI
SalI,SgrDI,NruI,MluI,SpeI,AseI
SspI,BciVI
PvuI,ScaI
FspI
AseI
AhdI
BciVI
DrdI
BspEI
BbsI
+4
+5
NheI
CD8 transmembrane
CD3 zeta domain
PfoI
PfoI
BamHI
KfiI
BmgBI
PsrI,AjuI
SmaI,BclI,BarI
MluI,BlpI,BstEII,BaeI,BsaXI,+4
TthIIII,BssHII,DrdI,RsrII,PflFI,TthIIII
PflFI
NotI,HpaI,XbsI
KasI,NarI,SfoI,PluTI
BbvCI
PasI
PciI,FseI
SacII
BfuAI,BspMI
BsaAI
SacII
AgeI
2500
cPPT/CTS
KfiI
+1
BsmI
BbvCI
FseI
NruI
Bs sHII
KasI,NarI,SfoI,+1
CspCI
NcoI
NdeI,BsaAI,SnaBI
SalI,SgrDI,NruI,MluI,SpeI,AseI
SspI,BciVI
PvuI,ScaI
FspI
AseI
AhdI

FIG.28